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MALE CELLS IN ANGIOSPERMS

I. SPERMATOGENESIS AND FERTILIZATION IN ASCLEPIAS CORNUTI

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(WITH PLATES I–III AND TWO FIGURES)

From the beginning of the study of fertilization in angiosperms up to the present time, so far as the writer knows, the existence of male cells with living cytoplasm has been a debated question.[1] Contradictory data about the same species have been reported, not only by different investigators, but sometimes by the same one. Such lack of knowledge has already been noticed (**8, 25**). According to the literature and my own investigations, therefore, the existence of male cells with living cytoplasm in angiosperms seemed to be improbable. Not considering this question solved, however, I continued investigating spermatogenesis in different representatives of angiosperms, paying special attention to plants indicated as having male cells.

The American plant *Asclepias Cornuti*, which has been studied many times from the embryological point of view, proved to be the most suitable for such investigations. As will be shown later, I found material of *A. Cornuti* which leaves no doubt about the existence in this plant of real male cells, formed just after the laying down of the cell plate in the body of the generative cell, and reaching the embryo sac in a state of perfect conservation. The principal deduc-

[1] To my great regret, not all the botanical literature of the last few years has been available for consideration.

tions of this investigation had already been announced at a sitting of the Society of Naturalists at Kiew in May 1919 (**9**), and at a sitting of the Ukrainian Academy of Science in May 1923, and also partly published (**10**). I regret that the present work, already begun in 1912, could not have been published sooner.

The material for the investigation has been collected partly in the Botanical Garden of the University of Kiew, and partly in the neighborhood of Kiew, where *A. Cornuti* is to be found here and there almost wild, and in considerable quantities. The latter material, being better pollinated, proved to be the more favorable for investigation. To secure a more rapid penetration of the killing agent, parts of the flowers were carefully prepared. After the floral envelopes had been removed, pieces were sectioned parallel to the direction of the course of pollen tubes, the ovaries being slightly dissected with a razor, the pollinia removed, etc.

The material secured during several periods of growth has been killed in different ways. The most apparently satisfactory killing agents were formalin mixtures, tried first by NAWASCHIN (**26, 27**), and afterward successfully used by other botanists of Kiew (**4, 21**). For the fixation of pollinia, the following mixture was used: 15 cc. of 1 per cent aqueous solution of chromic acid, 4 cc. of 40 per cent solution of trade formalin, and 1 cc. of glacial acetic acid. Pollen tubes and ovaries were killed with the same mixture, but in a weaker concentration. For this purpose the mixture was diluted with 17.5 cc. distilled water and the quantity of formalin reduced to half. Sometimes less concentrated solutions were used. Usually the objects were plunged in the killing mixture for 48 hours, during which time the solution was replaced once by a fresh quantity of the same composition.

When plunging the objects into the killing agent, measures were taken to make them sink. The results of the fixation of pollinia not only depend on the fixing reagents, but also on the age of the pollinia; old and rather dried ones do not fix well. Moreover, the unsatisfactory results of fixation, as it seems, are often due to other accidental causes, as the coming to the surface of the pollinia during fixation, their adhering to the walls of the vessel, and so on. That is why of two pollinia taken from the same portion one appears to be properly

fixed, while the contents of the cells of the other offer a homogeneous mass quite unsuitable for investigation. Care was also taken to let the fixing reagent penetrate directly to the pollen tubes.

Following fixation the material was washed in water for twenty-four hours, dehydrated, and imbedded in paraffin in the usual manner. By means of Yung's sledge-microtome, sections were prepared for the pollinia 5 μ, and for the ovaries 7.5, 12, and 13.5 μ thick.

The sections were stuck on the slides by means of albumen fixative. The pollinia often loosening from the glass, REGAUD'S method of covering the fastened and paraffin free sections with a fine collodion film was used (**19**). The sections of the pollinia were stained for the most part with Haidenhain's iron-alum haematoxylin, followed by a staining with 0.5 per cent aqueous solution of erythrosin.

A delicate differentiation of haematoxylin with a solution of iron-alum, and of erythrosin with alcohol, affords very good preparations for the study of the development of male cells in *A. Cornuti*. This method of staining proved to be less favorable for studying the contents of the pollen tubes, and quite unavailable for investigation of the process of fertilization. After applying different methods of staining, including Flemming's, recommended by investigators of the embryology of Asclepiadaceae (**12, 13**), I chose one of the mixtures of PIANEZE (**28**) consisting of "Malachytgrün," fuchsin S., nigrosin, saturated alcohol solution of copper acetate, and water. This method of staining, first applied to plant objects by FAWORSKY (**6**), seems unequaled when studying male gametes during their progress within the pollen tube and in the embryo sac, showing preparations quite transparent, on condition, of course, of a proper differentiation. The latter was produced first in alcohol, and finally a more delicate one in a mixture of one portion of alcohol with two portions of xylol. In some cases, preparations stained according to PIANEZE were differentiated once more, in the meantime being stained by a clove oil solution of orange G. This method also gave excellent results. Artificial germination of pollinia was also tried, but did not give any favorable results.

The development of male gametes in the representatives of *Asclepias* has already been studied specially by GAGER (**13**) and

FRYE (12), who treated them quite correctly as male cells. After the studies of KÖRNICKE (18), STRASBURGER (37), and NAWASCHIN (24), however, the existence of male cells with living cytoplasm in angiosperms was rather doubted, while on the other hand contradictory data about male gametes in *Asclepias* were given by GUIGNARD (15) and SCHÜRHOFF (31).

While GUIGNARD calls the male gametes in *Asclepias syriaca* L. (*A. Cornuti* Dec.) "generative nuclei," and draws them as such on his plates in pollen grains, as well as in pollen tubes, SCHÜRHOFF (whose paper I know only from MATOUSCHEK's report) suggests that the generative mother cell containing the two generative nuclei is maintained in *Asclepias;* that is, that here a binucleate generative cell is to be found. Moreover, many peculiar details in the process of formation of male cells in *A. Cornuti*, putting beyond all doubt the effectiveness of proper male cells in this plant, were not noticed in the papers of GAGER (13) and FRYE (12) regarding the same question. Accordingly, I consider reasonable the publication of the data of my investigation, in spite of the appearance at different times of various works treating upon the same subject.

As is known, the generative cell in *A. Cornuti* still lying near the wall of the pollen grain is hemispherical or lenticular (13, 36), depending somewhat on the shape of the pollen grain wall against which it lies (fig. 1). In the same stage the tendency of sharpening the ends of the generative cell is noticed.

As has already been reported for some other representatives of angiosperms, the generative cell in *A. Cornuti*, when withdrawn from the pollen grain wall, remains united with it by a thin band of cytoplasm (fig. 2). When detached from the wall it gradually assumes the shape of a more and more elongated spindle, with much extended tail-like ends (figs. 3, 4, 5), similar to what SMITH (35) observed in *Eichhornia crassipes*, appearing round in transverse sections including the nucleus, and oval in oblique ones. Similar cases must also have been seen by FRYE (12) in *Asclepias verticillata*, although he thinks their appearance due to the gradual change of the shape of the generative cell.

The cytoplasm of the generative cell is very dense, with minute granular structure, and, if quite normal and well fixed, does not in-

clude any vacuoles. In a pollinium with non-vacuolate generative cells, several cells happened to be found whose cytoplasm was somewhat vacuolated. Sometimes in the cytoplasm small bodies are observed. They may be stained black with iron-alum haematoxylin, and happen to occupy the place peculiar to centrosomes, but I never observed figures as reported by NAWASCHIN (**24**) for *Lilium Martagon*, which might have had even a distant resemblance to blepharoplasts.

In contrast with the cytoplasm of the generative cell, that of the pollen grain is much vacuolized. It usually contains deeply stained bodies of protein nature (figs. 3, 13, 18*a*), which have already attracted the attention of GAGER and FRYE, and have recently been described in detail by GUIGNARD. The two cytoplasms differ in their relation to stains, the generative being more erythrophyllous. If using iron haematoxylin stain, therefore, followed by an aqueous solution of erythrosin, the cytoplasm of the generative cell, stained red, would distinctly project on the somewhat blue ground of the pollen grain cytoplasm. The outer layer of the latter, however, quite distinct where in contact with the cytoplasm of the generative cell, is also stained a shade of red which is conspicuous in cases of the parting of the two cytoplasms from each other (figs. 5, 16). In the same way the upper layer of the cytoplasm of the pollen grain adjacent to the wall is often stained. I did not chance to observe a layer of the generative cell confining it from without and staining otherwise than the inner portion of its cytoplasm, this quite agreeing with the data of NAWASCHIN (**24**) concerning *Lilium Martagon*.

In treating the division of the generative nucleus, which takes place within the pollen grain, I find it reasonable to note that although *A. Cornuti* offers a helpful object for the study of the formation of male cells, it is quite unnecessary here to investigate the details of the nuclear division, especially because of the extremely small size of the chromosomes, which has been referred to in the literature (**13, 36**). For this reason I have studied only the chief features of the nuclear division of the generative cell.

During the growth of the generative cell, its nucleus gradually passes into the prophase of the division. This phase, in its early and later stages, has often been observed in my preparations, one of them

being shown in fig. 4. In this figure a somewhat curved generative cell is to be seen, with its nucleus in late prophase.[2] The generative nucleus, with still prominent outlines, includes a slightly stained nucleolus and twelve already individualized chromosomes, appearing in most cases as short and somewhat curved rods. It must be noted, however, that during the prophase two-limbed and U-shaped chromosomes were sometimes observed. After careful and extended counting, the number of chromosomes for the haploid nucleus agreed with GAGER and not with STRASBURGER (**36**).

More seldom I met with the metaphase of the division of the generative nucleus. This circumstance, as well as the rarity of mitotic figures, has already been noted by GAGER.[3] One of the cases of the metaphase that I have examined is given in fig. 5. One is impressed by the fact that the spindle threads do not occupy the whole generative cell, but only its middle part, and their focusing at the poles is scarcely defined; the much extended ends of the generative cell consist of granular cytoplasm. Such a structure during metaphase has often been found in my preparations.

That similar cases seem to have been observed by WYLIE (**39**) in *Elodea canadensis* is inferred from his figures, but nothing is said about it in the text. A complete contrast with the conditions I saw is presented by GAGER's data for *Asclepias Cornuti* and FRYE's (**12**) for *A. verticillata*, who figure generative cells in the metaphase stage, with the whole of their bodies occupied with filaments focusing at the poles and without any granular cytoplasm within. It is interesting to note that STRASBURGER (**37**) emphasizes this fact in his paper, where, referring to GAGER's figure, he says (p. 518):

> Eine andere Figur zeigt, dass in der generativen Zelle, die sich schon vor der Schlauchbildung verdoppelt, das gesamte Cytoplasma in der Spindelbildung aufgebraucht wird. Nur ein inhaltsleerer Raum umschliesst diese Teilungsfigur.

FRYE does not give any explanation of his figure, while GAGER states as follows (p. 138):

> The nuclear spindle in this division is different from those observed in any of the other divisions. It is rather sharply pointed at both ends, and much longer

[2] In the previous investigations of the spermatogenesis in *A. Cornuti* referred to nothing is mentioned about the prophase of the division of the generative nucleus.

[3] This is generally said about the division of the generative nucleus; in GAGER's paper only one figure of metaphase is given.

and more acuminate at one pole than at the other (fig. 32). The more tapering pole appears to be somewhat bent to one side, but this was doubtless caused by the shoving of the microtome knife, or by some other mechanical injury. The difference in the length and manner of tapering of the two ends does not seem to be due to the way the section was cut, as the poles seem well defined and pointed at both ends, and not truncate at one end as would be expected had the microtome knife cut off one pole. Here also the nucleus was too tiny to permit of the details of spindle formation, etc., being clearly made out.

Besides this difference of GAGER'S observations and mine, his words show that he closely approached the question of the structure of the ends of the generative cell, nevertheless attributing the little bend of one of its poles to an artifact, while the unequal tapering of both ends, in his opinion, does not depend on the direction of the slide. My observations proved that the question might be solved in a way somewhat differing from GAGER'S.

As already mentioned, during the growth of the generative cell its ends become very elongate, assuming a tail-like shape (figs. 4, 5, 12). These elongated ends wind and often dispose themselves in different planes. They are often cut off with the microtome knife from the middle portion of the generative cell, and, as they represent very fine cytoplasmic filaments, they are seen only if very well fixed and stained. This probably explains why none of the investigators of spermatogenesis in *Asclepias* mentions these tail-like projections of the generative cell, giving, as will be shown later, a peculiar shape to the male cells and sometimes rather obscuring the real structure of the male gametes. In rather rare cases when I happened to observe the generative cells in metaphase of their nuclear division, they usually were supported with an arclike outgrowth (fig. 5); but on comparing the earlier and later stages of development, I conclude that here both outgrowths must exist, the two being scarcely distinguished because of their winding.

Comparing GAGER'S data with mine, it may be thought that he figures the generative cell with its outgrowths cut off; whereas the longer and somewhat curved end of it mentioned by him is merely the base of one of them. In the stage of equatorial plate I counted 9–12 minute chromosomes, the splitting of which I did not succeed in seeing (fig. 5).

According to GAGER (13), during its nuclear division the gen-

erative cell is surrounded by a wall, persisting even later around the male cells. FRYE also reports similar walls in the pollen grains of *A. verticillata*, but according to his observations they are not always conserved to the end of the division of the generative nucleus. Upon comparing the figures of both writers with what I have seen in my preparations, I came to the conclusion that the walls are the external (membranous) layer of cytoplasm of the pollen grain adjacent to the body of the generative cell. Due to its staining similar to that of the cytoplasm of the generative cell (at least in the staining I used), and on the other hand differing from the rest of the pollen grain plasma, this layer is especially obvious in places, where because of the pressure the two cytoplasms are conspicuously detached from each other. If both cytoplasms are quite close to each other, this layer is more difficult to observe, if in general the observation be possible (figs. 1, 3, 4, 5, 8, 16). These causes and the difference in staining of preparations might probably explain the fact that FRYE observed the membranes round the generative cells at one time but not at another. Sometimes, if well stained, the outer layer of the pollen grain cytoplasm becomes very prominent, and makes the impression of a frame bordering the cavity which includes the generative cell or the male gametes resulting from it. This frame, in certain cases suggesting a cell wall, might deceive the investigator. Such cases are to be seen in GAGER'S figs. 32 and 33, and also in my fig. 16.

GAGER and FRYE do not mention the anaphase of the division of the generative nucleus. This stage appeared now and then in my preparations. As in metaphase, the middle part of the generative cell is occupied by spindle threads, the edges consisting of granular cytoplasm sometimes including separated vacuoles (fig. 9). In this figure it is also obvious that an outer protoplasmic layer in the central portion of the generative cell has also a granular structure. It must be noted that the fine structure of the cytoplasm of the generative cell may be seen distinctly if well fixed and stained. It happens that the generative cells of adjacent pollen grains of the same pollinium do not display equally the organization of their cytoplasm. Such a case is given in figs. 8 and 9. The first, being drawn from a casually overstained generative cell, does not give any idea of the real structure of its cytoplasm, appearing homogeneous.

During the anaphase of the division of the generative nucleus, I chanced to observe a very interesting phenomenon. In some of the generative cells I found the normal haploid number of chromosomes for *A. Cornuti*, that is, for two sperm nuclei twenty-four chromosomes separating toward the opposite ends of the cell, as is to be seen in figs. 6*a* and 6*b*, drawn from two successive oblique sections of a generative cell. In other generative cells in the same pollinium I counted half the normal number of chromosomes, that is only twelve chromosomes destined for the two sperm nuclei. Such cases are given in figs. 8 and 9, the uncommon distribution of the chromosomes about the whole cell being noticeable in the first one. Consequently two kinds of sperm nuclei may arise in a pollinium, one containing twelve chromosomes and the other six. As such mitotic figures are not of common occurrence I could not explain the cause of this event, but probably either a double reduction takes place, or during the separation the chromosomes fuse two and two. Probably the second supposition is right, their size being approximately twice the normal, which is noticeable in comparing figs. 6*a* and 6*b* with figs. 8 and 9, drawn to the same scale.

In my preparations the chromosomes of the anaphase have a peculiar appearance, often appearing two-limbed or even as if consisting of two globules with a little chink between them (figs. 6, 8, 9). Sometimes they were elongate or U-shaped, which is to be seen in figs. 7 and 8 (one of the upper chromosomes). Besides, it is obvious that the U-shaped chromosome in fig. 7 is somewhat narrowed in its middle part. As already stated, I have observed such chromosomes many times in the prophase. Sometimes they were so narrowed in their middle part as to seem to consist of two lightly oblong limbs. It is obvious that if the limbs are somewhat rounded, besides noticing a certain orientation of chromosomes in the body of the generative cell, the chromosomes can assume the shape observed in my preparations. This explanation agrees with GAGER's data suggesting the chromosomes of *A. Cornuti* as U-shaped, and figuring them sometimes with enlarged ends.[4] It is interesting that according to SAKAMURA's (**29**) fig. 9, even much longer U-shaped chromosomes

[4] It will be noticed from his figures that GAGER observed such chromosomes, not in the division of the generative nucleus, but during the division of the pollen mother cells.

of *Vicia Faba* happen to assume altitudes reminding one of the appearances in the anaphase of the generative nucleus division in *A. Cornuti* already referred to.

Passing to the telophase stage, I shall emphasize the fact that this stage is accompanied by an interesting process of formation of the cell plate, which from my point of view is of great importance in reference to the question of the existence of male cells in *A. Cornuti*. During the progress into the telophase, the cell plate always becomes more and more prominent, being especially well seen between the sperm nuclei passing into the resting stage (figs. 10, 11). Fig. 11 shows a somewhat later stage of the formation of male nuclei, as may be concluded from their larger size and the prominence of their nucleoli.

The cell plate was constantly observed during the late telophase, but, as this stage seems to be of short duration, the number of possible observations was not great. As shown in figs. 10 and 11, a well pronounced, though very delicate, cell plate is laid down in the middle of a typical barrel-shaped fragmoplast, consisting of quite fine spindle threads. In the first of the mentioned figures the fragmoplast is spread across the whole generative cell, while in the second one it is narrower than the cell. Here, as in the earlier stages of the division of the generative nucleus, the fragmoplast does not occupy the whole generative cell. Male nuclei, being mostly spherical and considerably less in size than the generative nucleus they are derived from, soon after their formation enter into the resting stage. Each of them includes one nucleolus, their chromatin appearing at this time as granules of different sizes. It results from my observations, therefore, that the ability of the cytoplasm of the generative cell of *A. Cornuti* to assume a fibrillar structure during its nuclear division, occupies an average position between the abilities that GAGER (fig. 32) and FRYE (fig. 25) state for 12 representatives of the same genus, and the data of NAWASCHIN (**24**) for *Lilium Martagon*, where no fragmoplast is formed.

Before discussing the formation of male cells, I shall mention with more detail the question of laying down of the cell plate, which precedes the formation of male gametes, not only in *A. Cornuti*, but also in some other representatives of seed plants. No data have been

found on this subject by the investigators of spermatogenesis in *Asclepias*, although in FRYE'S (**12**) paper there is a figure (fig. 26) similar to my figs. 10 and 11, but no traces of any cell plate are shown; and nothing is said about it in the text. FRYE'S figure generally seems much schematized, and does not give any idea of the rather subtle structure of the generative cell during the laying down of the cell plate. Very scanty data concerning the question under discussion are to be found in literature. Not only in angiosperms but even in gymnosperms the laying down of the cell plate before the formation of male gametes very seldom happened to be observed, the process being of rather short duration. The previously known cases may be divided into two groups: either the formation of the cell plate is not followed by an appearance of separate male cells, or the latter appear as quite independent structures.

Among the gymnosperms, *Pinus austriaca* belongs to the first group. In this species, according to FERGUSON (**7**), "No cell wall is ever formed, and in only one instance was a condensation of the spindle threads in the region of the cell plate observed." This is also illustrated by NAWASCHIN and FINN (**25**),[5] and in *Picea excelsa*, where MIYAKE (**20**) observed the appearance of the cell plate which afterward disappears (see also NAWASCHIN and FINN).

Among the angiosperms, *Lilium Martagon* can be referred to a certain grade in the same group, although NAWASCHIN (**24**) suggests that generally no fragmoplast is formed in the cytoplasm of the generative cell; yet STRASBURGER (**37**) did observe it in some cases with a rather weakly developed cell plate. To the same group must also be referred *Neottia Nidus-avis*, where, according to MODILEWSKI (**21**), in the plasma included between the sperm nuclei, a laying down of a fragmoplast with a delicate filmlike evanescent cell plate is observed; and probably *Ruppia* also, where the division of the generative nucleus is followed by a laying down of a rather fine cell plate, as was shown by MURBECK (**22**) and GRAVES (**14**).

There are still fewer representatives of seed plants for which data enough exist to place them in the second group. *Taxus canadensis*, studied by DUPLER (**5**), may of course be referred to this category.

[5] It is of interest to note that the fragmoplast with the cell plate in the figure of FERGUSON is similar to that observed in *A. Cornuti*.

DUPLER states that during the division of the body cell, "A broad spindle is formed, and the cell plate laid down on it is lenticular in outline, resulting in the formation of the two unequal male cells, a small lens-shaped cell, and a larger more rounded one."

As to the angiosperms, no definite data concerning this question have been given for any representatives, which has partly been noted by NAWASCHIN and the writer (**25**), and, so far as I know, we do not find in recent work any information about the laying down of the cell plate before male cell formation in angiosperms (see WYLIE **40**, SCHÜRHOFF **31**, **32**, HERRIG **16**, **17**, SAWYER **30**, TISCHLER **38**, and SHARP **33**).

A. Cornuti, therefore, appears to be the only angiosperm which can be assigned to the second group, for doubtless cell plates are repeatedly found before the formation of male cells. This process is perhaps of much more common occurrence among seed plants, but it has escaped investigators for various reasons.

After the cell plate is laid down, the separation of male gametes begins, a light appearing in the middle of the generative cell, revealing a slight chink between the newly formed male cells (figs. 17*a* and *b*, 12, 13). The division of the protoplast of the generative cell begins before the last traces of spindle fibers have disappeared; it takes place in the line of laying down of the cell plate, a denser wall, as it seems, not being formed. This will be discussed later. Usually I happened to observe cases where the separation of male cells had already occurred, but sometimes I succeeded in observing the actual process of division of the protoplast of the generative cell into two male gametes. A similar case is represented in fig. 17*a* and *b*, drawn from two subsequent sections of a generative cell. While in the first (*a*), where we have the uppermost portion of the cell, the parting protoplasts are still joined together by a fine cytoplasmic strip, in the second (*b*) they are quite independent.

Generally the surfaces of the separating male cells facing one another are not identical, one of them being often somewhat convex, while the other is concave (figs. 12, 13, 17*b*). Sometimes, however, these surfaces are corrugated (fig. 14*a*), and, if badly fixed, they become covered with jaggs and cavities, which give an impression that one male cell has drawn out one or many protoplasmic jaggs of the sister cell.

Male cells are almost mature when in the pollen grain, and with their tail-like projections they suggest bullheads or sperms of some organisms, as seen in figs. 12, 13, 14*a*, 17*b*, 18*a*, and 19. Comparing these figures, it is obvious that the shape of sperms varies within certain limits, perhaps partly depending upon their age. The broadened portion of the male cell, corresponding to the head of a spermatozoid, is either nearly spherical, or heart-shaped, pear-shaped, hornlike, etc.; in several cases it becomes narrow-tailed at once (figs. 12, 19), while in others it gradually assumes this shape (figs. 18*a* and *b*). In considerably elongated male cells sperm nuclei also assume an oblong form (figs. 13, 18*a* and *b*). The tail-like projections of sperms, showing, as already noted, much extended ends of the generative mother cell, vary somewhat, especially differing in their length and size (figs. 12, 18*a*, 19).

Sometimes male cells assume a very queer shape, as for instance the left one in fig. 18*a*, resembling a hammer-headed shark, or the right one in the same figure, twisted under the nucleus. In some cases the separation of male cells takes place when the generative cell is still slightly curved (figs. 12, 13), while in others (fig. 17*a* and *b*) it occurs when the generative cell is curved in a bow. Sperms when formed often settle, however, forming different angles (fig. 14*a*); sometimes they curve to such a degree that the middle portions of their bodies become almost parallel, and the ends, lying one upon the other, form a kind of closed arc. Such a case is shown in fig. 18*a* and *b*, where one adjusting of the microscope shows the sharpened ends of male cells (*a*), while the other (*b*) gives the impression of an almost closed arc. A more interesting case is fig. 16, where the tail-like projections lie so strictly one upon the other that by different adjustings of the microscope their ends cannot be distinguished, while only one definite adjusting shows a scarcely visible chink, showing this arc to be a result of the two tail-like projections lying one upon the other. When at the beginning of my investigation I saw this preparation, I thought that a binucleate generative cell, very narrowed in its middle portion, would be formed, but further study showed that this conclusion was wrong.

The variety of the observed cases increases because the enlarged parts of male cells, as if corresponding to the head of a spermatozoid, are sometimes supplied with beaklike outgrowths, and, if lying one

upon the other, an arched vault appears between the gametes (fig. 14*a* and *b*). The same male cells are represented by different adjustings of the microscope; by the first the male cells are quite independent (*a*), while by the second (a beaklike projection appearing by the upper cell and reaching the lower one) they seem to be reunited with a streak of male cytoplasm (*b*). If simultaneously much extended ends of male cells overlie one another, this combination results in a kind of ring. Nevertheless, it could be noticed in all the preparations that male cells become individualized after their formation while yet within the pollen grain. I have not met with the solid connection between the male cells observed by WYLIE (**39, 40**) in *Elodea canadensis* and *Vallisneria spiralis*, and by SHATTUCK (**34**) in *Ulmus americana* (see NAWASCHIN and FINN **25**). These cases of change in shape and disposition of male cells do not exhaust all the diversity of appearances which are to be seen, but they give a sufficient idea of it.

The cytoplasm of male cells preserved in my preparations the structure peculiar to the cytoplasm of the generative cell, but occasionally somewhat enlarged male cells appeared, with a vacuolated cytoplasm. These sperms have an extraordinary aspect, their cytoplasm reminding one of the cytoplasm of pollen grains (fig. 15).

GAGER'S (**13**) and FRYE'S (**11, 12**) papers include no data which would give a clear idea of male cells in *Asclepias*. The best figured by GAGER is in fig. 33, representing, as it seems, male cells with badly secured cytoplasm in its transverse section, almost like my fig. 20. On the contrary, male cells of *Vallisneria spiralis*, figured by WYLIE (**40**), are very like the corresponding elements of *A. Cornuti*, but deprived of the long outstretched ends so typical for the sperms of the latter.

Occasionally some original figures were found, one of them being given in fig. 16. Here male cells, under the influence of the fixing reagent, have shrunk and parted from the cytoplasm of the pollen grain. Between the male cells a partition is seen, which stains with erythrosin rose. This partition, by its ungranular structure and mode of staining, is very like the thinnest limiting layer of the pollen grain cytoplasm, closely adjacent to the cavities, including the male cells. Figures similar to the latter are somewhat like those that

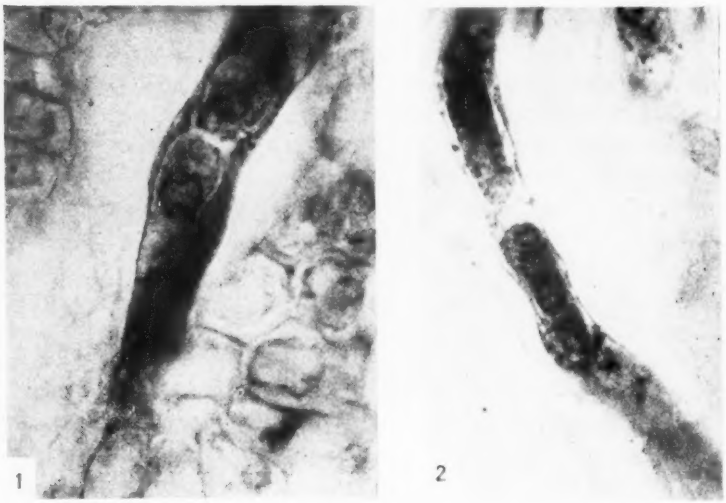
CHAMBERLAIN (**1, 2, 3**) has observed during the spermatogenesis in *Dioon edule*, *Ceratozamia mexicana*, and perhaps in *Stangeria paradoxa*. According to him, the spermatozoids of these cycads are formed within the two cells (mother cells) resulting from the division of the body cell. After the breaking down of the partition between the two spermatozoids and the wall of the body cell, the spermatozoids are set free.

As to the partition sometimes observed between the male cells in *A. Cornuti*, I have no direct evidence of its genetic relation with the cell plate appearing, as we have seen, in the telophase of the division of the generative nucleus. More credible appears the suggestion, based on the study of numerous preparations, that the partition represents a very fine membrane of the plasm of the pollen grain, which has penetrated between the male cells after their separation. At a considerable shrinking of male cells the membrane becomes especially prominent, as seen in fig. 16. Whatever may be the shape and disposition of male cells, they remain close together while within the pollen grain, so that their conjunction conserves the outlines of the mother generative cell, as shown in figs. 4, 12, 13, and others.

Much more difficult is it to observe well conserved male cells within the pollen tubes, but I succeeded in obtaining a series of preparations, mostly stained according to PIANEZE, which made possible the study of the details of the structure of the male cells during their progress within the pollen tube. Here they remain either close together, a fact already noticed by GAGER, or they follow after one another at a more or less considerable distance, as shown in figs. 21, 22, 23, and text figs. 1 and 2. In the first case sometimes there is no layer of pollen tube cytoplasm between the male cells (fig. 21 and text fig. 1). Sometimes sperms, during their progress through the pollen tube, are separated from one another at a considerable distance, as shown in fig. 23, where the second gamete, which is not figured, has become much separated.

In *A. Cornuti* I did not meet either the considerable variations in size, or any connection between the male cells, which WYLIE (**40**) observed in *Vallisneria spiralis*. In *A. Cornuti* the growth of the sperms during their progress through the pollen tube is rather insig-

nificant. Sometimes I happened to observe the presence of the tail-like projections of the male cells (fig. 23), in such cases the latter being very like swimming male gametes of some organisms. The tail-like end is either turned backward (fig. 23), or it may be in front. In the cases where the male cells are close together, their much extended ends are directed to opposite sides, as in pollen grains.



FIGS. 1, 2.—Photomicrographs: fig. 1, portion of pollen tube proceeding through ovarian chamber (pollen tube is in corner, formed with placenta and funiculus); male cells quite close together; portion of upper one not in section; on lower right, placenta tissue; on upper left, tissue of integument; fig. 2, portion of pollen tube within upper part of style; male cells at little distance from one another, their protoplasts also obviously limited from pollen tube cytoplasm.

My preparations do not suggest the least doubt that the bodies destined for fertilization in *A. Cornuti*, during their progress through the pollen tube, are real male cells. The cytoplasm of the latter, conserving in the pollen tube its small granular structure and its peculiar relation to stainings, differs from the cytoplasm of the male cells when within the pollen grain only in somewhat more accentuated vacuolization. In successful preparations, stained according to PIANEZE, the cytoplasm of male cells, somewhat loosened from the

plasma of the pollen tube, is conspicuous by its light red staining of the dark pollen tube contents (fig. 21 and text fig. 1). When staining with iron-alum haematoxylin, followed by the staining with an aqueous solution of erythrosin, one sometimes succeeds in staining the male cells a deeper red than the cytoplasm of the pollen tube (from such a preparation are made fig. 22 and text fig. 2). The latter appearing coarse grained, conserves to a certain degree its friable reticulate structure, which makes it differ strongly from the small grained cytoplasm of male cells (fig. 22).

It is interesting that, in GAGER's (**13**) opinion, traces of the delicate membrane of the generative cell remain for some time around the male cells while within the pollen tube. Evidently he is speaking of the finest limiting layer of the cytoplasm of the pollen tube closely adjacent to the male cells, as previously discussed. During the progress of male cells within the pollen tube, their nuclei remain in the resting stage, perserving the spherical shape, the nucleoli, and the chromatin in granules (figs. 21, 22, 23, and text figs. 1, 2). The tube nucleus, spherical within the pollen grain, becomes somewhat elongated while within the pollen tube, either preceding the male cells, or following them (fig. 22).

For a long time I could not succeed in observing the male cells in the moment of emergence of the pollen tube contents into the embryo sac, or even the next moment after it. One of the causes of this failure is the short duration of the events of fertilization, with the exception of the very fusion of the copulating nuclei, the latter having drawn already the attention of NAWASCHIN (**23**) and later of WYLIE (**40**).[6] Moreover, the considerable quantity of discharged material from the pollen tube, having a special affinity for stains, prevented the obtaining of good, well differentiated preparations, in which one might distinguish the male gametes from the remaining contents of the pollen tube and embryo sac. Occasionally I observed a male gamete within the pollen tube directly after the penetration of the latter within the embryo sac (fig. 24). Before fixation one of the ovules had been intersected so as to leave only the micropyle pole of its embryo sac; this made all its contents fall out, where-

[6] In order to retard the events of fertilization, WYLIE washed the ovules of *Vallisneria spiralis* in ice water and fixed them with a very cold killing agent, this method giving excellent results.

as the tip of the pollen tube, already within the embryo sac, remained undisturbed. As seen in fig. 24, the tube has already burst, and has evidently discharged a considerable portion of its contents into the embryo sac. It became possible, therefore, to observe the sperm stuck within the tube, having the aspect, not of a naked nucleus, but of a complete cell.

After a long series of failures, I succeeded once in observing male gametes soon after their penetration into the embryo sac, by using PIANEZE'S staining followed by the staining with clove-oil orange solution, differentiating with alcohol and its mixture with xylol. The cytoplasm of male cells stained red was definitely seen on the light green ground of the emerged contents of the pollen tube. In fig. 25 the sperms are seen lying quite near to one another, as is the case in the pollen grains or sometimes in pollen tubes. Owing to the fact that here the male cells still lie one upon the other, their union might be mistaken for a binucleate cell, if the history of the development of male gametes in *A. Cornuti* were not known. The male cytoplasm preserves its peculiar structure here also. It is to be noticed that, if stained according to PIANEZE, its capacity to stain red does not change, while the aptitude of the cytoplasm of the pollen tube for staining green increases after entering the embryo sac.

It is a matter of interest that, while the male cytoplasm of *A. Cornuti* stains very well, in some species of *Juglans*, studied by NAWASCHIN and the writer (**25**), it remained colorless; the latter being already noticed by WYLIE (**40**). In vain does he suggest, however, that in *Juglans* the presence of sperm cytoplasm, surrounding male nuclei within the embryo sac, has not been exactly proved. He is led to this conclusion by the fact that in our drawings the structural presence of sperm cytoplasm is not represented; in place of it a clear space is left. On account of it I feel obliged to explain that in our paper in several places it is stated that the sperm cytoplasm in the studied species of *Juglans* is homogeneous, and, by modes of killing and staining used, remains colorless, so that it is represented in the drawings as a clear space.

I did not observe the penetration of sperm cytoplasm into the egg cell, as did not the other investigators of the sexual process in angiosperms, WYLIE included (**40**), so that this question and its re-

lated problems remain unsettled. The male nuclei in *A. Cornuti* preserve their spherical shape, their nucleoli, and chromatin in granules in the embryo sac. They have a similar structure immediately after the penetration of male cells within the embryo sac, and also later, directly before the fusion with the female nuclei (figs. 25–28). GAGER (**13**) also figures the male nuclei of *A. Cornuti* in pollen grains and pollen tubes similar to those observed in my preparations (figs. 33, 35, 36, 37), but his paper gives no information about the structure of sperms while within the embryo sac. It is quite incomprehensible, therefore, what FRYE (**12**) saw in the embryo sac of this plant. In fig. 48 of his paper an embryo sac of *A. Cornuti* is seen in the very moment of double fertilization. One sperm flattens against the nucleus of the egg, while the other is close to the antipodal polar nucleus. Although these sperms are bereft of all traces of their own cytoplasm, FRYE calles them "male cells," and states that "the male cells are crescentic in form, reticulated with granules, and not conspicuously unlike in size." Striking is the great difference in structure and shape of sperm nuclei figured by FRYE, and those which I observed in the embryo sac of the same plant. The "male cells" of FRYE, according to his drawing, are very like the vermiform sperm nuclei of some Liliaceae (see, for example, NAWASCHIN **23**), only being of considerably smaller size. As these, they are deprived of nucleoli, their chromatin being reticulate.

According to my observations, the second sperm nucleus generally fuses with the fusion nucleus (figs. 26–30), whereas, according to the data of FRYE, it unites in most cases with the antipodal polar nucleus.

The existence of true male cells, therefore, before the formation of which a cell plate is laid down, seems to me quite proved in *A. Cornuti*. The existence of male cells in this species, and also in *Vallisneria spiralis*, makes their occurrence among other angiosperms quite probable; this should be determined by further investigation. In connection with the new data, secured during the last decade, the scheme appended to the publication of NAWASCHIN (**25**) and of the writer, figuring the male gametes of seed plants, must be somewhat changed and completed by including male cells among the sperms occurring in angiosperms.

Summary

1. From the beginning of the study of fertilization in angiosperms, the question of the existence of male cells with living cytoplasm has remained in doubt.

2. True male cells exist in *Asclepias Cornuti*.

3. During the telophase of the division of the generative nucleus, a very delicate cell plate is laid down in the middle portion of the fragmoplast. The latter occupies only the middle portion of the generative cell, whereas its much extended ends consist of granular cytoplasm.

4. The separation of male cells occurs where the cell plate is laid down.

5. Male gametes reach almost maturity while within the pollen grain, and being supplied with tail-like projections suggest in their general aspect bullheads or spermatozoids of some organisms.

6. The male cytoplasm differs considerably in its structure and relation to stains from the cytoplasm of the pollen grain, being therefore well delimited from the latter.

7. Following their formation, male cells remain in the pollen grain, although quite close to one another, but yet not united, contrary to what has been described for some representatives of angiosperms.

8. The tail-like projections of male cells arise from much outstretched ends of the generative cell.

9. Within the pollen tube male cells sometimes remain close to one another, and sometimes separate a more or less considerable distance, conserving their peculiar structure and relation to stains.

10. The tail-like projections of male cells are conserved while within the pollen tube.

11. Male gametes just after their penetration into the embryo sac maintain in their structure the unchanged cytoplasm. According to PIANEZE, the latter stains red, while the cytoplasm of the pollen tube emerged into the embryo sac stains green.

12. Like the other investigators of the sexual process in angiosperms, the writer did not succeed in observing the penetration of male cytoplasm in the egg.

13. The sperm nuclei, from the moment of their formation up to

their fusion with the female nuclei, conserve their spherical shape and resting stage. The writer has never observed them to be vermiform, as shown in FRYE's fig. 48 (**12**).

14. The existence of male cells on the one hand in *Asclepias* and on the other in *Vallisneria*, as has recently been shown by WYLIE (**40**), makes very probable their occurrence among other angiosperms.

15. In connection with the new data secured during the last decade, the scheme appended to the paper of NAWASCHIN and of the writer (**25**), figuring male gametes of seed plants, must be somewhat changed and completed by including male cells among the sperms in angiosperms.

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EXPLANATION OF PLATES I–III

All figures were drawn on the level with the microscope table, with the aid of an Abbé camera lucida. Nos. 22, 24, 25, and 26 with a Zeiss apochromatic imm. objective 3 mm., ap. 1, 3, and compens. ocular 6; nos. 4 and 18 with a Koristka apochromatic imm. objective 1.5 mm. and compens. ocular 6; nos. 6, 7, 8, and 9 with a semiapoch. imm. objective of Koristka $\frac{1}{15}$ and compens. ocular 8; all other figures with the semiapoch. imm. objective of Koristka $\frac{1}{15}$ and compens. ocular 6.

PLATE I

FIG. 1.—Portion of pollen grain, showing generative cell still affixed to pollen wall and tube nucleus; protein bodies within pollen grain cytoplasm.

FIG. 2.—Portion of pollen grain, showing generative cell parting from pollen grain wall.

FIG. 3.—Portion of pollen grain contents; generative cell soon after parting from wall; cytoplasm of pollen grain, showing spindle-shaped protein bodies.

FIG. 4.—Portion of pollen grain containing generative cell with nucleus in late prophase; twelve chromosomes seen; tube nucleus on left.

FIG. 5.—Preparation similar to fig. 4, but nucleus of generative cell in metaphase; upper tail-like end of generative cell lying upon tube nucleus.

FIG. *6a*, *b*.—Two successive, somewhat oblique, transverse sections of same generative cell with nucleus in anaphase stage: *a* showing nine and *b* showing fifteen chromosomes.

FIG. 7.—Oblique section of generative cell with its nucleus in anaphase.

FIG. 8.—Portion of pollen grain, showing generative cell in longitudinal sec-

tion and tube nucleus; anaphase of nucleus of generative cell, showing peculiar disposition of chromosomes through whole cell.

FIG. 9.—Portion of pollen grain, showing longitudinally dissected generative cell with nucleus in anaphase; as in *fig. 8*, number of chromosomes is half the normal, their size being approximately double that shown in figs. 6*a* and *b*, where number of chromosomes is normal.

FIG. 10.—Portion of pollen grain, showing nucleus of generative cell in late telophase, and tube nucleus; fragmoplast with obvious cell plate is seen; ends of generative cell, consisting of small granular cytoplasm, are partly cut off.

FIG. 11.—Stage of division of generative nucleus, similar to that shown in fig. 10, but fragmoplast does not occupy whole diameter of generative cell.

PLATE II

FIG. 12.—Male cells immediately after their formation, surrounded with pollen grain cytoplasm.

FIG. 13.—Preparation similar to fig. 12, but male cells more elongated, tail-like outgrowths not visible; sperm nuclei also oblong; close by tube nucleus a group of protein bodies; the figure being made from a well fixed preparation, neither are male cells loosened from pollen grain cytoplasm, nor is latter loosened from pollen grain wall.

FIG. 14*a*, *b*.—Same male cells by different adjustings of microscope: *a*, wholly separated male cells with streak of cytoplasm of pollen grain between their enlarged ends; *b*, foremost portions of same male cells; owing to appearance of beaklike outgrowth at upper sperm cell, male gametes apparently united by strip of male cytoplasm.

FIG. 15.—Male cells with vacuolated cytoplasm, showing extraordinary appearance; bit of pollen grains cytoplasm squeezed between male cells.

FIG. 16.—Portion of pollen grains, showing male cells with protoplasts shrunk and parted from pollen grain cytoplasm; ends of male cells much extended, lying one upon the other, forming a kind of arc somewhat enlarged in middle; between enlarged parts of male cells and their outstretched ends, lying one upon the other, a bit of pollen grain cytoplasm is squeezed; on left a group of protein bodies.

FIG. 17*a*, *b*.—Two successive longitudinal sections of generative cell in moment of separation of male gametes: *a*, individualizing protoplasts of male cells still united with strip of generative cell cytoplasm; *b*, separated male cells; in both a bit of pollen grain cytoplasm passing upmost in a fine strip is squeezed between the male cells.

FIG. 18*a*, *b*.—Same male cells by different microscope adjustings, showing their fantastic shape, right cell being bent under nucleus; enlarged fishtail-like ends are close together; opposite ends of male cells, formed from much extended ends of generative cell, overlie one another: *a*, whole pollen grain and its connection with vicinal pollen grains (outstretched ends obvious); *b*, same overlying one another, giving impression of continuous arc.

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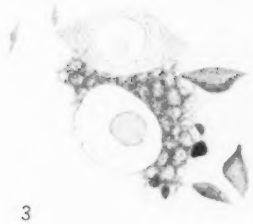

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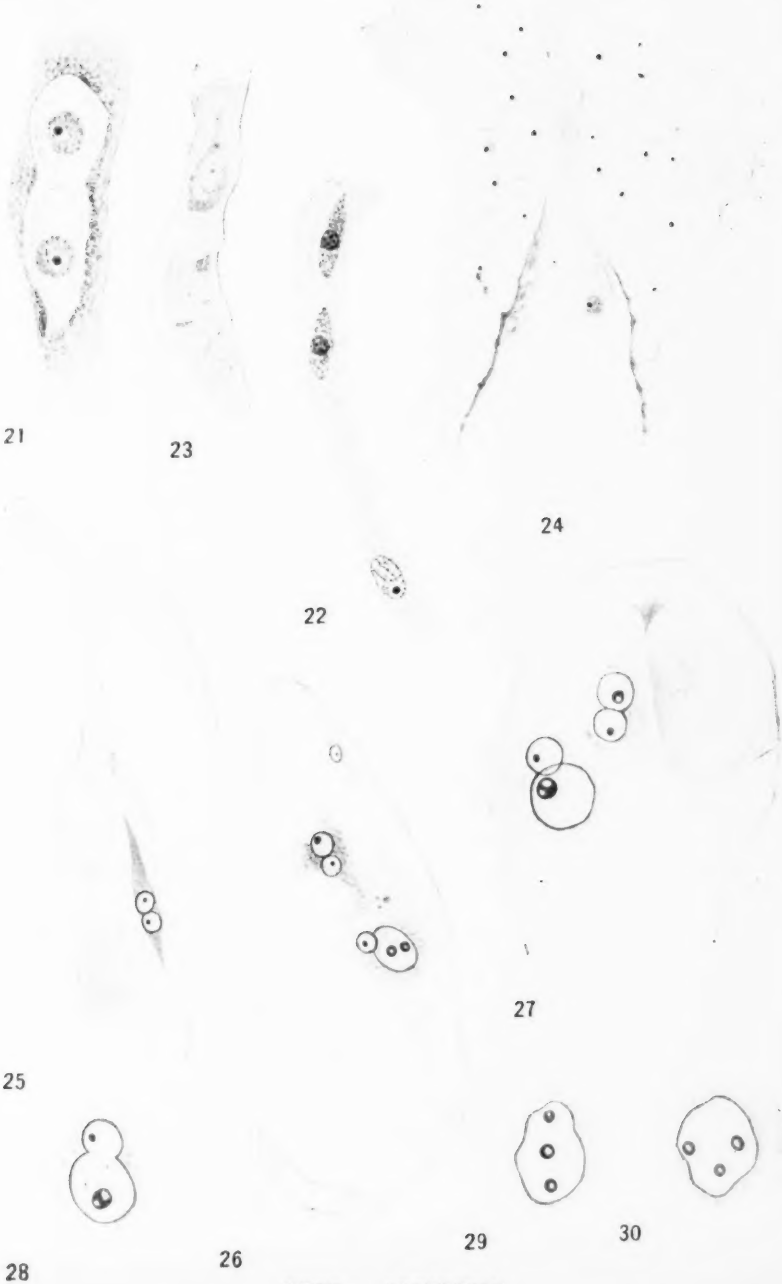
FINN on ASCLEPIAS

FINN on ASCLEPIAS



FINN on ASCLEPIAS

FIG. 19.—Male cells with tail-like outgrowths lying one upon the other with pollen grain cytoplasm around them.

FIG. 20.— Male cells in cross-section, with cytoplasm of pollen grain around and between them.

PLATE III

FIG. 21.—Portion of pollen tube passing within ovary on level with fastening of fourth ovule, counting from above; adjacent male cells separated merely by small space from one another and from pollen tube cytoplasm (lower one nearly all in section, portion of upper being cut off).

FIG. 22.—Portion of pollen tube within upper part of style; in front tube nucleus; male cells more separated than in fig. 21.

FIG. 23.—Male cell with tail-like end directed backward within portion of pollen tube passing in upper part of ovary.

FIG. 24.—Micropylar pole of ovule; contents of embryo sac fallen out except pollen tube penetrated within; latter is perhaps already burst out; male cell is stuck at its end.

FIG. 25.—Micropylar pole of ovule with pollen tube contents just emerged into embryo sac; on right male cells are close.

FIG. 26.—Embryo sac in act of fertilization; one sperm nucleus penetrated within egg and in close connection with its nucleus, second just approaching fusion nucleus; on upper right of egg the nucleus of one of synergids, lower antipodals.

FIG. 27.—Oblique section of upper portion of embryo sac, same stage of fertilization as in fig. 26; uppermost, on right of egg, emerged contents of pollen tube.

FIG. 28.—Fusion of sperm nucleus with fusion nucleus.

FIGS. 29, 30.—First nuclei of endosperm formed by triple fusion.

THE GAMETOPHYTE OF LYCOPODIUM CERNUUM IN HAWAII[1]

OTTO DEGENER

(WITH PLATES IV–VII AND TWO FIGURES)

Hawaii National Park, but a few hours' ride from the city of Hilo, is situated at an elevation of 1200 m. on Mauna Loa, an active volcano. Here, within an area of 10–20 sq. km., may be found every type of habitat, ranging from that of the bare glassy flows of "pahoehoe" lava in the crater of Kilauea, or the Kau Desert, to that of the humid tree-fern forest. Near the brink of the crater itself is a series of earthquake crevices from which steam continuously rises. This extends about 1.5 km. from a region of volcanic ash, upon which a few stunted plants of a peculiar Hawaiian composite and of a heath manage to exist, to an area thickly covered by an almost impenetrable tangle of vegetation. The conspicuous species characteristic of the latter luxuriant type of vegetation are the tree *Metrosideros polymorpha* Gaud.; a small tree-fern of the endemic genus *Sadleria; Gleichenia dichotoma* Hook., which clambers over any support to a height of 30–45 dm.; and *Lycopodium cernuum* L., which forces itself up through the underbrush and frequently rises to a height of 15 dm.

In December 1922, the writer, searching for the gametophytes of club mosses, noticed several sickly plants of *Lycopodium cernuum* at the brink of a stream crack in the area known as the Sulphur Bank. Upon observing these plants more closely, a few small sporelings were discovered on the sloping sides of the crevice. With this clue as to the type of locality in which the gametophyte of the species might be found, similar situations were investigated. Thousands of young sporelings and gametophytes were discovered, but since the circumstances under which these plants were growing can be duplicated only in a region of volcanic activity, stations not influenced by subterranean heat will first be described.

[1] Contribution from the Department of Botany of the University of Hawaii.

Stations under normal conditions

Six isolated groups of sporelings and gametophytes were found growing under ordinary conditions within 1.5 km. of the Volcano House. Here the vegetation is similar to the luxuriant type just mentioned. The mean yearly rainfall is approximately 104 inches. A brief description of these individual stations will be pertinent to a question to be discussed later.

1. At the road that leads around the rim of the crater, workmen had dug out some weathered lava, leaving a hollow in the roadside several decimeters in diameter. Here seven sporelings, most of them only a few millimeters in size, were growing among fine moss.

2. On either side of a sunken woodroad leading to a tree-fern forest, more than a score of specimens were found. They were growing near the base of a sloping embankment of clay soil 5 dm. high, made by the roadcut. Most of them were in company with young mosses. An indication of the relative rapidity of growth of the gametophyte of this species, in contrast with that of *L. clavatum* and *L. obscurum*, is shown by several plants which were growing at a spot where it was very plain that the wheels of carts had sheared off the soil in passing. This station seemed drier than the station previously mentioned, and was exposed to full sunlight. Most of the plants a centimeter or more in height were dying off at the base. The younger plants appeared stunted and not thriving.

The four remaining stations were located along a railroad bed abandoned years ago by loggers. The rails had been removed, but the ties remained and had partly decayed. Their surfaces were generally level with the soil and covered by a sparse vegetation of moss and grass.

3. One of these stations was a very damp slope below an overhanging rock, and was exposed to the early morning sunlight. The few specimens found, which were commonly in the protophyll stage, were loosely imbedded in moss.

4. Another station was also on a slope, but along a shady stretch of the railway. Within an area about a meter in diameter, more than a dozen sporelings were found, ranging from a few millimeters in length to branching and rooting specimens several centimeters long. They were rooted on a bed of fine moss that had overgrown the gravelly embankment.

5. At another station a few sporelings 7 cm. or more in length were noted at the side of the roadbed in dry soil. These plants were dying, presumably from lack of moisture.

6. The last station discovered yielded by far the best material. It covered the entire width of the roadbed for a length of 10–12 m. The place was rather well shaded by the giant fronds of several individuals of a *Cibotium*, while shrubbery and thickets of *Gleichenia* were encroaching upon the roadbed from either side. The ground was unusually moist, since here the railway passed over a depression. The soil was very gravelly, due to volcanic ash, but nevertheless tenaceous, because of an admixture of tawny clay especially rich in aluminum and iron. It was generally covered by a fine mat of young moss of coarse texture, while a sedge (*Uncinia Lindleyana* Kunth), a grass, and a few small ferns were the predominating vascular plants. In the center of the roadbed, where but few of the larger plants had gained a foothold, about seventy-five gametophytes with sporelings possessing from three to a dozen leaves were gathered. Away from the center small sporelings were conspicuously absent, whereas larger ones that had branched profusely and had rooted at the nodes were the most outstanding plants.

In May 1923, a station was discovered near Honolulu, on the island of Oahu, on a wind swept ridge at an altitude where precipitation is heavy. About fifty gametophytes and sporelings were collected on a sloping moss covered embankment less than a meter in diameter. Four sporelings several centimeters in length were growing near the edge of the station, with small grasses; all the other specimens were much smaller and grew where no larger vascular plants had developed.

The method of growth of these sporelings is not that which is typical of the adult sporophyte. The first branching is initiated before a height of 2 cm. is reached (pl. VII). The resulting limbs of the dichotomy diverge almost at straight angles to one another. One branch, however, is usually slightly more vigorous than its fellow. After this first branching, a modified dichotomy simulating monopodial growth becomes apparent. Here the branches are spaced 1–1.5 cm. apart. This type of branching usually continues until the main axis attains a length of about 6 cm. The terminus then tends to push into the soil, to swell at the tip, and to take root. Even

before a root has developed, however, a shoot arises from the whitish swollen part. It commonly makes an acute angle with the main stem and continues growth in the same direction. In this manner the plant creeps for a considerable distance, the space between successive rootings becoming longer and longer.

At each of these nodes, adventitious roots, along with several shoots, soon develop, and the latter tend to sprawl over the ground in a manner similar to that of the main rhizome. One or two of these shoots commonly become detached from the main axis. This tendency to break into isolated plantlets is more noticeable at the place of origin of the sporeling. It is usual for the part directly developed from the gametophyte to die, while a few of the adjoining segments commonly die also. The remainder have a very sickly appearance, the part between the rooting "nodes" having a tendency to die first. Because this weakened condition of the sporophyte increases the nearer its point of origin is approached, it is usually difficult to trace a sexually produced plant to its source, even though the sporeling be but a few decimeters in length.

The advanced sporophyte does not exhibit such short segments, nor does it appear weak like the developing sporeling. Instead, it creeps for a considerable distance over the ground before rooting. Then a shoot, developing at the point of anchorage, rises almost vertically for 10–25 cm. before branching. One branch then continues the plagiotropic growth so characteristic of the plant, and finally roots again. The other branch of the dichotomy grows erect to a height of a meter or more in favorable situations, such as are common near the volcano, and gives off a series of smaller branches that dichotomize more or less regularly. Upon the ends of the higher ones the strobili are formed. Their habit of hanging no doubt suggested the specific name. In vigorous fruiting individuals the branching is so regular that in counting the ultimate number of branches developing at different heights on the upright stem a perfect geometrical progression is discovered.

Stations under volcanic conditions

Most of the steam fissures are less than 15 dm. across, although some may even reach a width of 45 dm. They are commonly less

than 3 m. long because their sides have partly caved in, but some are at least 35 m. in length. Fig. 1 gives an approximate idea of the location of the crevices, the smaller ones being omitted. Pl. IV, however, shows a large part of the area in which the crevices are common. These extend from the hotel and the Volcano Observatory, shown in the distance, to the foreground and beyond. If all the

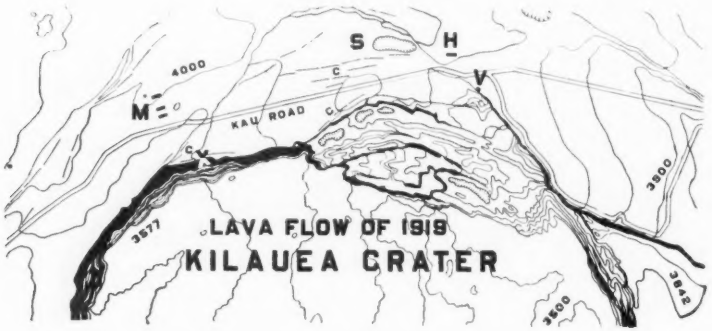


FIG. 1.—Map of volcanic region adapted from U.S. Geol. Survey, showing steam crevices in which gametophytes of *L. cernuum* are common: *C*, volcanically heated crevices; *H*, Volcano House; *M*, Kilauea Military Camp; *S*, Sulphur Bank; *V*, Volcano Observatory; *X*, volcanic station depicted in pls. II and III; Scale, 1 Km. = 30 mm.

steam fissures of that general region were placed end to end, their length would total more than 2 km.[2]

The perpendicular sides of these crevices are formed almost solely of consolidated clinkers. For many decimeters from the top (the distance depending upon the character of the fissure and the amount of escaping vapor) the sides are clothed by different algae and mosses. These, intermixed at the base with a reddish clay, form a covering sometimes as much as 3 cm. in depth. It is upon this substratum that gametophytes and sporelings of *L. cernuum* grow in enormous numbers.

In practically all crevices inspected, certain striking facts were noted. Where the heat is greatest, as in the deeper part of the fissure, plants are entirely absent. Farther toward the surface, where the

[2] Many of these steaming crevices are already obliterated with rubbish, for lack of a more convenient place to put it.

temperature is about 40° C., a very dark sooty layer of different blue-green algae is common, the predominating one being *Fischerella thermalis* (Schabe) Gomont. This area then grades off quite definitely into one in which very dwarf mosses predominate, none of which was found in the fruiting stage. They are commonly overgrown by a thick gelatinous mass of *Gloeocapsa*. Finally, at the brink of the crevice, vascular plants become noticeable, comprising chiefly an association of *Gleichenia dichotoma* and *Lycopodium cernuum*. These plants (of which all but the largest specimen shown on pl. VII are from the crevices) always increase uniformly in height the farther they are located from the heated crevice. On the perpendicular side of the crevice itself, the lycopod specimens were imbedded in countless numbers in the layer of mosses and slimy algae. Because of the light green color of the sporelings they stood out very conspicuously, studding the moss bank every few centimeters. Not one of these sporelings on the vertical wall of a steam crevice was found to be over a few millimeters in height. Most of them had developed scarcely anything except protophylls (fig. 2), or were buried for the greater part of their height in the substratum. The temperature at their base attained a maximum of 35°, but where they grew in greatest numbers and apparently under the most favorable conditions, the thermometer stood at about 31° C. At 26° the gametophytes were rare and sickly, but sporelings a centimeter or more in length were very common. The latter were always weak and much branched, often dying in places, or even entirely dead.

Thus there was a band two or more decimeters in width, studded with gametophytes with their minute sporelings, on the smooth vertical wall of a uniformly heated crevice. Where the temperature was around 31° the plants were spaced but a few centimeters apart in many cases; at a little higher temperature they would become less numerous; while above 35° not a single specimen was found. Similarly, they were less numerous where the side of the fissure was cooler than 31°, while at 26° the few that occurred appeared less thrifty than under slightly warmer conditions. In areas still cooler than 26° the gametophytes were comparatively rare, but much larger sporelings had grown from them.

In another crevice under almost identical conditions, a small

part of the perpendicular wall protruded for several centimeters in the midst of a bed rich in gametophytes and minute sporelings. Immediately above this ridge all the sporelings were much larger, and had actively branched, but none of them extended beyond the protecting ledge into the rising steam. The general character of these plants, however, resembled that of the flora of cold, wind swept

FIG. 2.—Sporelings of *L. cernuum* typical of warmer areas of volcanic steam crevices; ×6.3.

alpine regions. This same type of growth was observed in several other crevices where protection from the steam was similarly afforded. It may thus be inferred that the further growth of the sporelings in these fissures is inhibited by the hot vapor.

On a level rocky surface, in a spot that was covered by volcanic ash and unprotected from the sun, a small amount of vapor was noticed escaping from a crack 5 cm. long. On the leeward side and

hence exposed to steam, a tuft of moss was growing in which thrifty gametophytes and small sporelings were imbedded. From the position of these specimens it is plain that full exposure to sunlight is not detrimental. The reason why gametophytes in non-volcanic stations are so limited to shaded areas would therefore seem to be due, not to any ill effect of direct sunlight, but to the resultant desiccation of the surface soil to which they are confined. Stations are commonly situated on embankments, whether shaded or not, probably because such a vertical surface is not as likely to dry as is a horizontal one. Moisture that collects in the soil during rainy weather tends to seep out slowly. This not only keeps the side of the embankment moist during periods of drought, but insures good drainage during a wet season. On the other hand, a horizontal surface will more readily dry out during a short period of fair weather. That sunlight is not detrimental to the growth of the gametophyte and young sporeling, is further evidenced by the fact that the writer was able to keep typical plants growing for six months in direct sunlight without any noticeable ill effects. They were grown, however, under glass in an almost saturated atmosphere.

Pl. V at *X* shows a station of gametophytes and sporelings at the very brink of the crater of Kilauea, while the gradual slope of Mauna Loa is seen dimly in the distance. This station is unusual, yet it illustrates well the occurrence of the lycopods under heated conditions. In this case the steam escapes from the side of the precipice at no great distance from the plants. The soil upon which they are growing is heated mainly from below, but the entire plants at the edge of the crater are also frequently exposed to the hot vapor. Pl. VI is a closer view of the same plants. The match (*A*) nearest the brink of the precipice marks the spot where the thermometer registered 35° C. Here neither gametophytes nor sporelings were growing. Where the second match (*B*) stands, the temperature stood at a little more than 33°. Here were found many gametophytes and many minute sporelings; the latter are plainly visible in the photograph. The pencil (*C*) marks the position of a thick tangle of large sporelings. The thermometer here showed a temperature of 29°. The relationship between the temperature and the size of the sporelings is likewise exhibited in the typical crevices. Above approximately 35°

no lycopods can grow. At a slightly lower temperature they are extremely common but invariably small, usually little more than in the protophyll stage. At a still lower temperature almost all the sporelings are large, spindly, and much branched. Where the effect of the heat is negligible, the sporelings may be missing entirely, as is shown in this photograph. Or, if larger sporelings are present near the edge of the heated station, they appear not to have developed in that immediate locality from gametophytes, but rather to have spread by means of runners from the volcanic stations.

At the time that these photographs were taken (August 1923), these crevices were further investigated. In part of one crevice, from which a bed of moss several centimeters in thickness containing gametophytes and sporelings had been removed seven months before, several prothallia of different ferns were observed, among which were three gametophytes of *L. cernuum*. Two of them had sporelings with three protophylls attached; while from the third, a fourth leaf was just developing. If we exclude the possibility of minute gametophytes washing down to that spot from the moss above, it is practically certain that these three specimens developed to that stage within little more than half a year. This is not surprising in view of the occurrence, previously described, of specimens growing on the roadside that had been scraped by a passing vehicle.

Some of the crevices at that season were so dry that the mass of *Gloeocapsa* had dried into a firm gelatinous skin. Upon this many gametophytes were seen that varied from almost microscopical size to occasional specimens 7 mm. long and 5 mm. wide. From other heated localities were taken moderately dry layers of fine moss free from *Gloeocapsa;* here, in area of only 4 sq. cm., fourteen unconnected gametophytes and sporelings were gathered. In another clump of moss that was of looser texture, scores of minute gametophytes were gathered merely by tearing the moss to pieces in the field with a penknife. Most of these were about half a millimeter in diameter, and spaced 2–3 mm. apart. All appeared to be entirely green. They were situated at a depth of about 5 mm. in the moss where it had just turned brown. Whether the conditions for the development of the gametophyte are as favorable in the mass of *Gloeocapsa* is not known. Comparatively few gametophytes without

sporelings were collected with this blue-green alga, although the minute sporelings were extremely common.

In comparing these volcanic stations with the stations located under normal conditions, it might be thought that the great profusion of specimens in the former was entirely due to the moisture laden atmosphere. This, however, does not seem to be true. On the contrary, the presence of heat is the major factor for the prolific development of gametophytes and minute sporelings. Proof of this is afforded by the presence of the specimens in greatest numbers in the crevices where the substratum is heated to a certain definite range of temperature, while negative evidence is afforded by the uniform moisture conditions of the entire crevice and of its rim. The whole surface of such an area drips with moisture. The air is also saturated, so that in a few minutes one is covered with minute droplets of water. In some localities it is impossible to collect specimens because of the unbearable heat of the air, which, escaping from certain parts of some fissures, is blown by the wind from one place to another. If moisture were the only factor involved in this particular case, the entire area should have been covered uniformly with specimens. This by no means implies that volcanic heat is required by the gametophyte. So far as moisture is concerned, the conditions are probably the optimum, and are constant, but the specimens are not found in such profusion because of the moisture, but rather because of the exceptionally high temperature. In the non-volcanic stations, on the other hand, lack of moisture is probably the factor that kills off the gametophytes most commonly. We are justified in concluding, therefore, that the gametophyte and the minute sporophyte have an extraordinarily high optimum temperature and a very high optimum moisture requirement.

It is with design that the term "minute sporophyte" is used, since, as already stated, only small sporelings were found in such exposed volcanic stations. Wherever sporophytes above a few millimeters in length were found in the steam crevices, they were invariably protected from the greater part of the heated vapor. In spite of this protection these sporelings were commonly dying. Only such as were near the edge at the top of the crevices, and had been able to creep away from proximity to them, had continued their growth

and were developing into mature sporophytes. In spite of the great number of gametophytes under heated conditions, the larger sporelings were far less common there than in non-volcanic stations, where the gametophyte was rare. It should be remembered that around both types of station *Gleichenia* was very common, and formed a close association with the lycopod. Since in GOEBEL'S (4) own words, "die Gleichenien zu den an offenen, zeitweise trockenen Standorten wachsenden Farnen gehören," we can apply the same remark equally well to the mature sporophyte of *Lycopodium cernuum*.

These facts justify the conclusion that the older sporeling and the mature sporophyte have an optimum temperature requirement that is similar to that of the average flora of the region. They also have a lower optimum moisture requirement and a very low minimum moisture requirement, both considerably lower than the corresponding moisture requirements of the gametophyte.

Historical

These facts lead to certain interesting speculations, but before considering them it will be well to refer to some of the literature pertinent to gametophyte ecology.

An early report (1827–28) that has a bearing upon the present problem is by BLUME (2). He describes what might be considered a variety or form of *L. cernuum* as *L. vulcanicum*. This plant, he writes, "Crescit ad margines craterum montium ignivomorum Javae." In 1841 SPRING (10), in his monograph of the lycopods, observes of *L. cernuum* that, "La localité des îles des Açores, sur le parallèle de Lisbonne est la plus boréale de toutes celles qu'affecte l' espèce. Le *L. cernuum* y a été trouvé, suivant une communication que je dois à M. Gay, par Guthnik, voyageur des naturalistes à Esslingen, autour d' une source chaude, sur un ancien volcan de l' île de Pic." A similar report was made by BERKELEY (1) in 1857. He states that "A curious instance of the appearance of a tropical species in temperate latitudes is afforded by *L. cernuum*, a species very widely diffused in the tropics. It occurs about the warm springs of the Azores in Terceira and St. Michael's, in spots exposed to the sun, and again in the southern island of St. Paul, a fact which has its parallel in the occurrence of a tropical *Pteris* under similar circum-

stances in a small island in the Mediterranean." To this is appended the following footnote: "The ticket which accompanies the specimens in the Hookerian Herbarium states that the temperature of the water was 114°, the air at the same time being at 65°." These temperatures in centigrade would read about 46° and 18°.

In 1884, TREUB (**11**) published his work on the sexual generation of *L. cernuum*. In spite of his exhaustive researches, he gives but a brief account of the type of locality in which the gametophyte of the species thrives. As is well known to those who have collected the sexual generation of plants of this genus, the mature sporophyte and the gametophyte of the non-epiphytic lycopods are rarely found in the same type of locality. It is therefore especially to be regretted that a fuller description of his stations was not included. TREUB sowed the spores of several lycopods upon tree trunks in the garden at Buitenzorg, Java, and met with considerable success in obtaining prothallia. He raised gametophytes of *L. cernuum* in his room, by sowing the spores on a kind of clay upon which he had noticed the prothallia growing in the natural stations. In a later publication (**12**) he mentions the fact that he was able to gather hundreds of gametophytes of *L. cernuum* in April. He attributes the abundance of these plants at that time to the excessively heavy rains, which, falling in the west of Java in January and February of that year, probably facilitated the germination of the spores. Not until his discussion (**12**) of the endophytic fungus does he allude to the type of station which he has found to be best adapted to the development of the gametophytes. There he gives the character of his stations in the following words:

> Sans vouloir dire de ce Lycopode qu'il fuit les terreins riches en humus, il est certain qu'il croit de préférence sur un sol dépourvu de détritus végétal. J'ai récolté les prothalles et les plantules qui m'ont servi dans mes recherches, sur des terreins ou, le plus souvent, il paraissait ne pas y avoir de couche de humus du tout. Une espèce de terre glaise rougeâtre, fréquente dans les environs de Buitenzorg, est ici la station préférée par le *L. cernuum*.

In 1898 RACIBORSKI (**9**), speaking of *L. curvatum* Sw., and giving as the synonym the name "*L. vulcanicum* Blume Enum. 266; *L. curvatum* Sw. Spring, 81; *L. cernuum* var. *curvatum* Baker 23," reports it from Java as being "Ein Erdfarn des nicht stark beschat-

teten Waldbodens und der baumlosen Stellen der unteren mittleren und oberen Gebirgszone zwischen 1400–3000 M. Eine charakteristische Kraterflanze der Vulcane Java's, aber auch weit von den Kratern wachsend. Salak, Gedé, Pangerango, Telaga, Bodas, G. Guntur."

That same year KERKHOVEN (**8**) reported the species growing at about 1.5 km. and upward on the lava slopes of Guntur, one of the active volcanoes of western Java. *Gleichenia* is also reported as growing to man's height in the neighborhood of the crater.

In 1916 HERTER (**5**) mentions this lycopod as being found in the Bismarck Archipelago of German New Guinea, "In der Nähe der Solfataren, auf erhitztem Boden auf dem Vulkan Ghaie . . . . , in der Nähe von durch Schwefeldämpfe erhitzter Lava. Häufig und gesellig, charakteristisch an und um den Krater des Vulkans, selbst auf neuen Lavamassen, aus deren Spalten Schwefeldämpfe kommen." For New Britain he reports its occurrence as "Krater bei 'Die Mutter.'"

Not until 35 years after TREUB's discovery do we get another record of a find of gametophytes. It is to HOLLOWAY, who has worked upon the sexual generation of the New Zealand lycopods, that we must turn for data that seem to have most application to the ecological studies discussed in these pages. He states that *L. cernuum* "grows very abundantly throughout the northern part of the North Island of New Zealand on clay moorlands. . . . . It thrives especially in North Auckland amongst scrub vegetation of the *Gleichenia-Leptospermum* association, individual plants often attaining to a length of 12 to 15 ft., and the upright branches to a height of 1–4 ft. It is also extremely common on the Volcanic Plateau, in the neighborhood of hot water and near fumeroles" (**6**). He also states that *L. cernuum* is abundant on the clay gum lands of the Auckland Province, areas occupied at no great bygone period by the Kauri-tree forests, but "it shows still greater luxuriance in the neighborhood of hot-water streams."

> On many occasions, and in many different parts of the Auckland Isthmus, as also in the Mongonui County, I have noticed young plants of this species growing in the vicinity of older plants. The spores would seem to germinate very freely. But . . . . a dry summer would bring about the destruction of most of

the plantlets. A damp clay bank or a shaded roadside cutting in the neighbourhood of adult plants is the best place to search for the young plants and prothalli —i.e., they are more in evidence under artificial than under natural conditions. Such a clay bank, if damp, shaded, and old enough for a thin covering of moss and slime fungus to have appeared on it, invariably contains the young plants of this species, generally in great abundance. . . . . The very young plants and prothalli of *L. cernuum* are difficult to clean owing to the intimate penetration of the clay and slime by their numerous rhizoids.

Later, HOLLOWAY (**7**) states that "They were all dissected out of humus consisting for the most part of a decaying short moss." In referring to the many species that he has collected, an additional statement (**6**) is worth quoting:

From my own observations, extended over a good number of years and in many parts of New Zealand, I have found it to be an almost invariable rule that young plants and prothalli are not to be met with in localities in which the adult plants are abundant. It is only in special localities, such as a damp shaded clay bank or roadside cutting, or some other patch of recently disturbed soil in the neighbourhood of adult plants, that the young plants occur. But it must be added that when favourable conditions are present prothalli occur often in the great abundance. . . . . Where the land-surface was deeply covered by volcanic ash during the eruption of Tarawera in 1886, *L. cernuum* was growing in abundance some years ago near certain streams of hot water. Colonies of this description must have originated from spores, but, unfortunately, no exact data are available as to the first appearance of young plants.

Theoretical considerations

From a consideration of the stations discovered in Hawaii, and of the brief review of some of the localities from which this lycopod has been reported by other investigators, it is evident that three general types of stations occur. The gametophyte is found in two, and the sporophyte occurs in the third.

The type of station that must be the most common for the gametophyte throughout its range in the tropics is generally a moist clay embankment. Such an embankment is usually partly shaded, and often of comparatively recent origin, so that fine mosses predominate. A red clay composed largely of aluminum, iron, and silica seems to be preferred. This may be due to the fact that this lycopod requires large amounts of these elements, since its ash is composed of over 16 per cent aluminum and 30 per cent silica (**3**).

By far the most favorable station for the gametophyte, and the

minute sporeling that may arise from it, is limited to regions of volcanic activity. The substratum may be one of almost bare clay, or preferably a mat of fine mosses. This is frequently overgrown by a species of *Gloeocapsa*, which, if too thick, possibly tends to kill the lycopods. The latter are found imbedded in the moss or in the mass of blue-green algae. The atmosphere and the substratum are saturated with moisture and heated to approximately 31° C. The type of station especially adapted to the mature sporophyte, on the other hand, differs greatly from both types of stations adapted to the gametophyte. The sporophyte is found commonly in open glades and on the outskirts of forests, where it is frequently exposed to seasonal dry weather, so extreme as to be fatal to the gametophyte. It grows particularly well on volcanic soil, where it is often associated with *Gleichenia* in forming almost impenetrable thickets.

We are now in a better position to understand why the mature sporophyte of *L. cernuum* is not found in stations in which the gametophyte is common, and vice versa. It is because the mature sporophyte, as already explained, has an optimum temperature that is similar to that of the average vascular plant in its vicinity, and lower than the optimum temperature for the gametophyte. It has a lower optimum moisture requirement than the gametophyte, and, in particular, the capacity to endure a low minimum moisture condition. The conditions that make the locality particularly fit for the one stage of development, therefore, are quite unsuited to the other. It is for this reason that the gametophyte and the mature sporophyte are not found growing in the same habitat.

That this difference in the moisture requirements between the gametophyte and the thickly corticated sporophyte should obtain, is easily understood from a consideration of the delicate nature of the sexual generation, but why such a great difference in the heat optima should occur can only be conjectured. Nevertheless, the facts may justify a tentative suggestion.

If we accept the antithetic theory of the alternation of generations in plants, we must consider the gametophyte as the primal generation, from which the sporophyte has gradually developed by the intercalation of a post-sexual generation. Now if we take the two alternate generations as but one individual plant, which in a

certain sense it is, we are forced to concede that the gametophyte is the earlier part formed.

If there be any truth in the trite statement that ontogeny recapitulates phylogeny, we cannot consider this solely from a morphological viewpoint. It is the inherent qualities of the living protoplasm that function, or express themselves, according to a definite sequence, as formulated by this law; and it is the result of this progressive ontogenetic change in the protoplasm that is so easily perceived in the morphological changes. Since the latter is but the indirect effect, it is really the physiology that should be considered first in a study of the morphological ontogeny. If the physiology of a plant varies according to its stage of development, it is evident that the environment best suited to it must also be different. Thus if the plant is in an early stage of its development, the conditions under which it will grow best might well be similar to those to which its far-off ancestors were habitually exposed.

The striking structural contrast of gametophyte and sporophyte easily explains their very diverse habitats, as BOWER has shown. The prothallus is almost hydrophytic, requiring a continuous and abundant supply of water, although living on land. It is so delicate that it cannot withstand the effect of dry conditions for any length of time. It has neither roots for the absorption of water from below the surface of the soil, nor a vascular system to conduct this water through its tissues. Furthermore, it is absolutely dependent upon water for the liberation of its sperms and the fertilization of its eggs. The morphology of the sporophyte, on the contrary, points to an almost xerophytic habitat. It has a very thick cortex that inhibits the transpiration of moisture; it possesses roots for absorption and a complex vascular structure to aid in the conduction of water through its body; and it is dependent upon dry conditions for the liberation and dispersal of its spores. But are we not in reality considering the effect instead of the cause? It were better to state that the filament growing from the spore is limited, not adapted, to conditions of moisture because of its inherent physiological qualities. Its progenitors, of a type that were comparable with this stage, were presumably threadlike forms. The thalloid gametophyte, now somewhat altered because of the symbiotic fungus, is limited to a semi-

aquatic habitat, because its phylogenetically comparable ancestors grew under similar conditions. What is then more likely than that the minute sporeling just recently developed from the gametophyte should also be limited to conditions little different from those under which the sexual generation thrives best?

If, instead of the antithetic, we advert to the homologous theory of the alternation of generations, this difference in the optima for growth in the different stages of development might be explained as follows. If the two generations were distinct from the beginning, the asexual stage might have been the more plastic; it could, therefore, in later times adapt itself far more readily than the gametophyte to the more prevalent ecological conditions.

The great profusion of gametophytes and small sporelings under conditions of abnormal heat and saturated atmosphere may then be due to the following fact. The conditions which are the optimum for the gametophyte and minute sporeling simulate the climate of that geologic period in which the progenitors (that never advanced ontogenetically beyond such a general state of development) found their optimum conditions for growth. On the other hand, the older sporophyte has diverged too far in its environmental requirements from the gametophyte to be able to grow successfully in a situation that is suited to the latter. The conditions that are necessary for the one stage have gradually become inimical to the other.

Should this divergence in environmental requirements between the gametophyte and sporophyte generations be true, we should be able to find similar cases among plants that stand on a similar level of phylogenetic development. A few instances which apparently support such a theory have come to attention.

About 75 m. distant from the *Lycopodium* stations, and on the other side of the perpetual firepit called Halemaumau, a long crevice about 2 m. in width and 3 m. in depth was found. This was situated near the Kau Desert, in the midst of bare lava, much of which had flowed to that vicinity not many years before. The bottom of this crevice had been filled in by natural means, so that it was possible to walk upon it. Scarcely any steam could escape, although the ground was intensely hot.

The side of the crevice showed stratification, the rock being

interbedded with sandy layers of ash. All was heated volcanically and was quite moist, although not saturated. In many places a whitish salt incrusted the surface. Upon one side of the crevice, a great profusion of *Psilotum triquetrum* Sw. and *Ophioglossum* sp. was growing. The former was the more common plant, and very often grew between the cracks in the rock layers; the latter was limited to the sandy layers only. Because of the isolation of this crevice in the midst of a desert of lava, there is little doubt that these plants developed in this situation by spore dispersal. Also, because of the great number of individual plants of *Psilotum* growing in isolated cracks in the rock, it is probable that this method of propagation was frequent. That the same was also true for *Ophioglossum* is probable, since many plants were growing in the layers of decomposing ash, isolated one from the other by the rock strata. Although *P. triquetrum* is very common in this general locality, and is adapted to the "*a-a*," or rough lava fields, the *Ophioglossum* sp. apparently is quite rare. Throughout the entire time that the writer collected plants in this region, only one other specimen was found. It was discovered the same day about 1.5 km. away, under an overhanging bank in a deep gully in the Kau Desert. The only other plant growing with this specimen was a species of *Marchantia*.

Although one cannot make a true comparison of these stations based upon plants belonging to different orders, yet since these plants possess certain characteristics in common, and appear to be the remnant of an archaic flora, a comparison is worth attempting. It will be noted that in the Kau station the conditions of the substratum, so far as heat is concerned, were practically identical with those of the volcanic lycopod station. The moisture content was also similar. In both stations the sexual generation appeared to have developed under unusually favorable conditions. Where the conditions were those of heat and moisture, the sporophyte of *Lycopodium* failed to develop beyond its earliest stages; while where the conditions of unusual heat and moisture were absent from the air above the Kau station, the sporophytes of *Psilotum* and *Ophioglossum* developed in a normal manner.

It must be remarked that neither specimens of *Psilotum* nor *Ophioglossum* were found in the volcanic lycopod station. In fact,

L. cernuum was the only vascular plant present. The subterranean nature of the gametophytes of these two plants may account for this absence. A saturated substratum, as found at the lycopod station, would prevent every access of air to them and quickly cause their death. The prothallium of *L. cernuum*, however, being at the surface of the ground, is always exposed to the air or in close proximity to it, no matter how saturated the substratum may be.

That not a single lycopod was found in the Kau station was surprising. One explanation is that since it is limited to growing at the surface of the soil, the conditions at this station were too dry. With a sandy substratum, exposed to the sun, and with no water vapor to supply moisture, this plant would readily dry out. Another possible reason for its absence may be the presence of salts at the surface of the soil. That the subterranean gametophytes of the other cryptogams would neither be exposed to drying, nor to that concentration of salts at the surface, probably accounts for their presence in this station.

That the sporophyte and gametophyte of the more northern lycopods should also be found in stations by themselves, would tend to weaken the view that a heated environment more nearly approaches that to which the ancestral plants were accustomed. Yet we should consider that the gametophyte of *L. cernuum*, not excepting that of *L. Selago*, is the most primitive type known for the genus. Nor would this instance militate against the theory of divergence in respect to other environmental conditions. It is a fact, however, that in its ontogeny the sporophyte becomes more and more adapted to an environment that is unfavorable, if not fatal, to the gametophyte from which it develops.

This very inadequate study may give the clue to a vastly greater problem, one upon which BOWER has fully touched in his writings. From about Middle Devonian time, cryptogams with a well defined sexual and asexual generation became common. Such plants then constituted the predominant land flora of the Late Paleozoic or Carboniferous, but by the time of the Mesozoic, practically all the more significant spore bearing plants of the former era were gone. The others, the ferns excepted, have gradually become

extinct, until but a few insignificant representatives of this archaic flora remain to the present day. It may be suggested that with the changing environmental conditions during the latter part of the Carboniferous, those plants that were the least plastic, from a physiological point of view, quickly disappeared. Those remaining tended to adapt themselves to existence on a drier terrain. In most cases one generation was unable to change its requirements for growth as quickly as the other. This resulted in a progressively greater divergence in their respective optima for existence. The sexual generation, however, being built upon a plan that is dependent upon moisture, found it more difficult to become adapted to a drier climate than the asexual generation, which, from its beginning, had been dependent upon relatively dry conditions for its chief function of spore dispersal. Thus we find that by the beginning of the Triassic, those plants that were still keeping in the race between their minimum requirements for existence in the sporophyte generation and the ever changing conditions of their environment, had picked up their lagging gametophyte generation. They carried it from the ground where it could no longer surmount the obstacles in the environment, and protected it within sporophytic tissues where a suitable artificially created environment was possible. Thus, in order to survive, the sporophyte generation had given protection to the gametophyte generation. Henceforth the latter became more and more dependent and progressively reduced, until in the present era the dominant angiosperms harbor barely more than the essential reproductive organs of the gametophyte. Of course, the foundation for these theoretical considerations is a very slight one; at least it may furnish a basis for further investigation.

Summary

1. On the island of Hawaii, and under normal ecological conditions, six stations for the gametophytes and small sporelings of *Lycopodium cernuum* L. were discovered; on the island of Oahu and under similar conditions another station was found.

2. On the sides of volcanic crevices near the crater of Kilauea, and under conditions of abnormal heat, thousands of gametophytes and small sporelings of *L. cernuum* were discovered.

3. The gametophyte of *L. cernuum* grows far better under volcanic conditions than under conditions without volcanic heat.

4. Exceptionally high temperature and abundant moisture are two of the optimum conditions for growth of the gametophyte of *L. cernuum*.

5. The conditions of heat and moisture that are best suited to the development of the gametophyte of *L. cernuum* are injurious to the development of the sporophyte.

6. Through a tentative theory of divergence, an attempt is made to account for the difference in environmental optima of the two generations.

7. Such a theory of divergence may explain in part the gradual extinction of the cryptogamic flora of past geologic eras.

This paper was begun at the University of Hawaii, in partial fulfilment of the requirements for the degree of Master of Science. The writer wishes to express his great indebtedness for encouragement and advice in the preparation of the manuscript chiefly to Dr. JAMES B. POLLOCK, former Exchange Professor from the University of Michigan at the University of Hawaii. For similar favors thanks are due to Dr. HAROLD S. PALMER of the latter institution, as well as to Professor A. VINCENT OSMUN of the Massachusetts Agricultural College. Through the kindness of Dr. T. A. JAGGAR, Director of the Hawaiian Volcano Observatory, it was possible to illustrate the paper with photographs of the stations.

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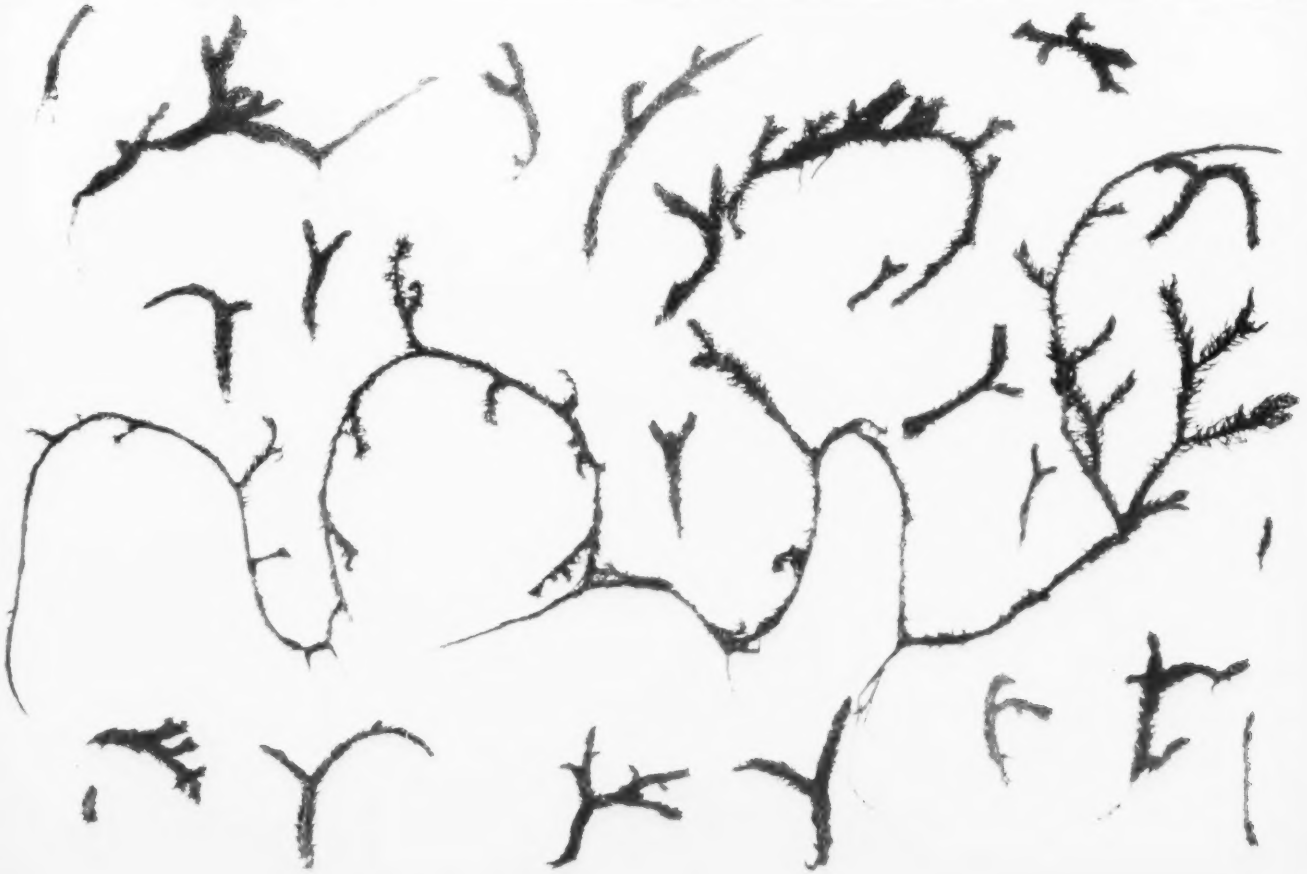

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EXPLANATION OF PLATES IV–VII

PL. IV.— Volcanic steam crevices in which grow thousands of gametophytes and sporelings of *Lycopodium cernuum*.

PL. V.— Station of gametophytes and sporelings of *L. cernuum* (at *X*) growing at brink of crater of Kilauea under abnormal conditions of heat and moisture, due to proximity to steam vent.

PL. VI.— Closer view of some lycopod station: at *A*, where lycopods are absent, thermometer registered 35°; at *B*, where gametophytes and only small sporelings are growing, thermometer registered about 33°; at *C*, where large much branched sporelings are very common and gametophytes very rare, thermometer registered 29° C.

PL. VII.— Larger sporelings of *L. cernuum* from stations in volcanic steam crevices or at their very brink (with exception of largest plant).

STRUCTURE OF THE RHIZOME OF EQUISETUM GIGANTEUM

ISABEL M. P. BROWNE

(WITH THREE FIGURES)

Introduction

The fragment of the rhizome of *Equisetum giganteum* L. on which the present observations were made was supplied by Professor R. C. MACLEAN of Cardiff, to whom I wish to offer my best thanks. The material consisted of a single node of the rhizome with considerable portions of the internodes above and below. The exterior of the rhizome was bluntly and obscurely angled, but there were no obvious ribs. The hardness of the external layer of the rhizome made the preparation of serial sections very troublesome, especially in the region of the node, and I was unable to avoid local tearing of the tissues.

The diameter of the axis in the internodes is about 5.5 mm., and that of the central cavity about 2.5 mm. At the organically lower end of the specimen the axis contains nine bundles and vallecular canals. Each bundle possesses a distinct endodermis or sheath. There is no general endodermis. In the internodal condition the bundles are separated by spaces about two and one-half times their own size. The outline of the bundle is elliptical, the longer axis being directed radially. The carinal canal is large, occupying about half the bundle; its greatest width may be in a radial or in a transverse direction. Usually hardly any remains of the protoxylem are to be seen in the carinal canals. The phloem forms an arc, slightly concave internally, at the periphery of the bundle. The lateral groups of tracheids sometimes abut on the ends of the arc of phloem, or they may be somewhat more deeply seated. The space between the arc of phloem, the lateral groups of metaxylem, and the carinal canal is occupied by parenchyma, of which the middle cells are relatively large, thin walled, and slightly elongated radially. Each lateral group of metaxylem generally contains four to six, sometimes only

two or three reticulately thickened tracheids. More often than not the middle tracheids of a group or band appear to be the largest, but there is no regularity in this. The tracheids may be arranged in a more or less regular band, usually uniseriate; or they may be disposed in a group in which there are locally two or three tracheids side by side. The vallecular canals are large and bluntly triangular. They are separated from one another by radiating bands of parenchyma, four to eight cells in thickness, which lie opposite the bundles. The blunt apices of the triangular vallecular canals run in between the bundles, reaching to about the depth of the outer limits of the carinal canals. They are separated from the carinal cavity by about four to eight rows of cells. The cortical tissue between the bundles and the radiating bars of cortical parenchyma separating the vallecular canals contains numerous starch grains, while the outer layers of the cortex consist of small cells, either devoid of or with very few starch grains. The outer layer of the rhizome possesses a thick brown cuticle, and its cells have dark, thick walls. The next layer consists of smaller cells, the walls of which are also strongly sclerized. This marked thickening extends to a third layer of cells, especially to their outer walls. Altogether there are six to nine rows of cortical cells between the epidermis and the vallecular canals. The cells gradually become larger and thinner walled toward the interior.

Nodal structure

As we pass organically upward in the series of sections through the relatively main rhizome, indications of the insertion of the stele of the branch begin to appear. As the branches are inserted between the bundles of the internodes below them, the outer and lower portion of the obliquely inserted ramular siphonostele is inserted on two lateral groups of metaxylem belonging to different bundles. It is noteworthy that the tracheids of the lateral groups of metaxylem on which the ramular siphonostele is inserted higher up assume nodal character before the tracheids of their fellow groups in the same bundle. GWYNNE-VAUGHAN (3) showed that in the aerial stems all the tracheids of the metaxylem have reticulately thickened walls, and this is true of the metaxylem of the rhizome. In the latter the elongated narrow metaxylem tracheids are gradually replaced,

as the node is approached, by more numerous short, wide tracheids of the usual nodal type and with a different kind of reticulum.

One of the preparations for the development of the branch is the division of the vallecular canal opposite into two by a somewhat wide band of parenchyma. The vallecular canals are so wide (at their widest more than 1 mm. across) that two small canals remain on either side of the bridge, even while the latter is being traversed by the stele of the branch. The ramular stele is nearly 0.5 mm. in

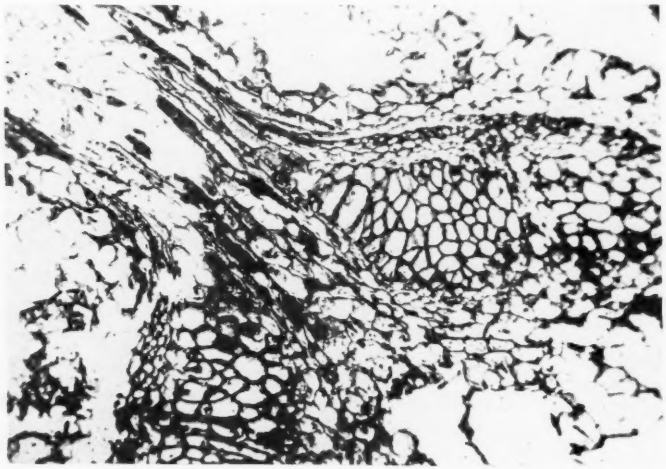

FIG. 1.—Transverse section of part of node of rhizome of *E. giganteum*, showing insertion of ramular stele; note narrow elongated tracheids (protoxylem?) inside ordinary nodal ones; near either edge may be seen xylem of leaf trace, one or two narrow elements, passing through nodal xylem.

diameter, and its xylem consists entirely of reticulately thickened tracheids. Most of these are of the usual nodal type. Some of the more internally situated tracheids, however, offer a strong contrast to the others. They are narrow, relatively elongated elements, with thin reticulations (fig. 1). It is possible that they represent protoxylem of a modified sort, for, as will be explained later, the axial protoxylem of the rhizome consists of somewhat similar elements. Such a differentiation between the tracheids composing the xylem of the siphonostele found at the base of all the branches

throughout the genus does not seem to have been recorded before, and does not occur in the steles of the branches of the aerial stem (BROWNE 2). These narrower tracheids belong definitely to the stele of the branch, and are not in connection with the axial protoxylem.

Three branches were initiated at the node in question. None of these had broken through the sheath of the parent axis; all of them had reached much the same stage of development. The vascular elements below the first diaphragm of the branch seemed to be fully differentiated. The tracheids were fully lignified, and in the pith below the diaphragm was a central group of dark brown cells. Although the diaphragm itself is already clearly indicated as consisting of several rows of short cells, the tissue above it is in a highly immature condition. Above this level no indications of vascular tissue can be distinguished, but the branches, which had hitherto grown out nearly at right angles to the parent axis, turned obliquely upward toward the organic apex of the latter. A little higher up in the series of sections the ochreola, or first and rudimentary leaf sheath of the branch, may be distinguished abaxially and laterally as a sheath two cells in width, just becoming free from the axis (fig. 3). Like the ramular siphonostele, the ochreola is inserted obliquely, the side away from the main axis being lower than the adaxial side. On the branches in question the ochreola was not yet discernible on the adaxial side.

All the branches gave rise to a pair of rootlets. These arose at opposite points on the sides of the branch, one root being at its insertion directed toward the organic apex of the main rhizome, and the other in the opposite direction. Their vascular supply is given off at exactly opposite points, is equal in amount, and has attained a similar degree of differentiation. In fact, everything points to the two roots of a branch being equivalent and contemporaneous in development.

Both roots eventually grow backward along the rhizome, that is, away from the organic apex of the main axis. Thus the steles of both roots, each with a sclerenchymatous sheath, appear obliquely cut at the edge of the sections through the main rhizome, before the insertion of the branches is reached. They are here seen passing

through tissue which has been shown in other species to belong to the leaf sheath. This tissue has become concrescent during ontogeny with the tissue of the main axis. So intimate is the concrescence that all boundaries between the two tissues have been obliterated, except at the point of separation of the leaf sheath from the axis. Here the junction of the free epidermis of the axis with that of the upper surface of the leaf sheath is marked by a double band of sclerized cells.

These dormant branches, or rather their apices, lie in the space between the parent axis and its leaf sheath. The concrescence between these two is continued longer opposite to the leaf traces, and these only pass out into the sheath at a level at which the branch apex is lying between the sheath and the main axis and has assumed a direction almost parallel to that of the latter.

In bundles preparing to give off traces the carinal canals usually become filled by a non-cellular substance staining deeply with Vesuvian brown, presumably by mucilage. In this substance may be seen the remains of tracheids of rather various sizes, adherent to the walls of the canal. Eventually, before the leaf traces are given off, the carinal canals disappear and their place is taken by short, wide, reticulately thickened tracheids of the nodal type, somewhat smaller than the more peripheral ones. Interspersed among these more internal nodal tracheids, which first make their appearance a little higher up than the other nodal tracheids, are two other kinds of elements. Firstly, there are occasional parenchymatous cells of much the same outline as the tracheids between which they lie. Intermediate between these parenchymatous cells and the ordinary nodal tracheids are elements in which the wall is already partially lignified, but the cell contents, although contracted, have not yet disappeared. Secondly, there are more numerous elements, also of the same shape as the tracheids, the lumen of which appears to be almost or completely obliterated, the cell contents being replaced by an opaque substance staining darkly with Vesuvian brown. At first sight these dark brown cells suggest small canals filled with mucilage, but by careful focusing the original tracheidal wall, stained by gentian violet, can be distinguished. If the same cell be followed through successive serial sections it can usually be seen

that a small central part of it is free from the brown substance. The latter appears to adhere to the wall, and, moreover, stains rather more deeply than mucilage. It is probably a kind of sclerized thickening, although no stratification is visible, at least in sections 14 μ in thickness.

At the inner edge of the nodal tracheids, or separated from them by one or two parenchymatous cells, are a few small elements of protoxylem. When these are cut slightly obliquely their walls appear to be reticulately and not spirally thickened. The bars of thickening are thin, however, and do not fork freely, so that in a longitudinal section of a tracheid the type of thickening is not very unlike the spiral form usually found in protoxylem. A few of the protoxylem elements in a median position pass through the axial bundle, to depart as the xylem of the trace. The reticulate thickening of these departing tracheids, of which there are usually two to four (never more than five), is well seen, since their long axes are directed obliquely outward. The traces remain minute throughout their course; nevertheless they appear to become concentric while in the cortex. After the trace has become free from the bundle it passes out very slowly, pursuing a steeply oblique course. As already mentioned, the portions of the axis opposite to the traces remain concrescent with the leaf sheath, after the latter has become free from the rest of its surface. Consequently the traces pass across parenchymatous bridges, and only enter the free portion of the leaf sheath rather more than half a millimeter above the level at which they detach themselves from the axial bundles. As soon as they have passed over the sheath becomes completely free from the axis. In the aerial stem the xylem of the trace was surrounded by a narrow parenchymatous sheath while it was passing through the bundle (**2**). In the rhizome there is no such definite sheath, but parenchymatous cells, some of which have the same orientation as the departing tracheids, are relatively common at the level of departure of the traces.

The vertical extent of the nodal xylem is much less than in the aerial stem previously described (**2**). In the sectors of the stele on which the ramular siphonosteles were inserted higher up, tracheids of a nodal character make their appearance relatively

early; that is, they usually make their appearance at from 440 μ to 640 μ below the level at which the dying out of the nodal or supranodal tracheids, lying vertically above traces that have departed, caused the reconstitution of the bundles in the upper internode. In one case, however, the distance between these two levels was only about 320 μ. In bundles on which no branch steles were inserted, the nodal tracheids usually made their appearance about 280 μ to 350 μ below the dying out of the nodal wood opposite a trace that had departed, although in one such bundle some nodal tracheids appeared as much as 420 μ below the level of the reconstitution of the bundles in the next internode. A few nodal tracheids persist a short time in the reconstituted bundles, dying out about 230 μ to 280 μ higher up. It must be remembered that, as is so often the case in *Equisetum*, the node is slightly oblique, so that there is no continuous ring of nodal xylem to be seen in any section. Indeed, two of the bundles failed to become connected by nodal wood, and remained throughout the node separated by a narrow tract of parenchyma. A similar example of reduction of nodal xylem has been observed in cone-bearing branches of *E. debile* Roxb. (1).

Not only is the nodal wood of the rhizome much reduced in quantity, compared with that of the aerial stem, but there are also indications that it develops rather late. The rhizome studied was fully mature, as is shown by the large size of the central, vallecular, and carinal cavities and by the considerable length of the internode. Yet the nodal wood contained a considerable number of undifferentiated or incompletely differentiated elements.

Another indication of reduction of the vascular system at the nodes is afforded by the discontinuity of the protoxylem in this region. Some axial protoxylem persists after the departure of the trace, but such elements, which are very small and somewhat crushed by the growth of the larger nodal tracheids, are usually scattered. In some cases the bundles, as at first reconstituted, appeared to contain a few elements of protoxylem continuous with those of the internode below, but usually the protoxylem died out before the formation of the separate bundles of the next internode. In any case, the reconstituted bundles soon assume a more or less circular outline, and it can then be seen that the larger tracheids fill up the

whole of the space included within the bundle sheath, except that occupied by the external arc of phloem. Eventually the bundle assumes the typical internodal form, the larger tracheids being replaced by parenchyma, except where they are replaced by the smaller tracheids composing the lateral groups of metaxylem. The more deeply seated large tracheids persist longer than the more peripheral ones. Protoxylem also reappears.

As compared with the aerial stem, the reduction of the xylem of the traces is much greater than that of the axis. In the aerial stems the trace contains, in addition to numerous elements of protoxylem, a considerable number of metaxylem tracheids. In the rhizome the traces possess no metaxylem, while the protoxylem may be reduced to two, and seldom consists of more than four elements. In the only specimen available, all the tracheids of the internodal lateral strands were completely differentiated. As the position within the strands of the smaller tracheids was variable, it was impossible even to conjecture whether the internodal xylem of these strands developed centripetally, as in the aerial stems, or centrifugally, as in the other axes, in which the development of the lateral strands has been followed.

Root bearing branches of rhizome

JANCZEWSKI (4) studied the development of the root bearing branches of the rhizome of *E. arvense* and *E. limosum*, and came to the conclusion that true rhizogenous branches only occurred in the latter species. He stated that the branches of *E. arvense* became dormant after giving rise to one (or at most two) roots, and he noted that where there were two roots the second was inserted above the first, on the lower side of the branch with reference to the organic apex of the parent axis. He seems, in fact, to have held the modern view that these root bearing structures of *E. arvense* are merely arrested branches. In *E. limosum*, however, he distinguished two sorts of buds: a small number, usually not more than one at a node, of relatively voluminous branch buds, destined to develop as aerial stems or branches of the rhizome, and a larger number of true rhizogenous buds. He admitted that the mother cells of the branch buds and those of the rhizogenous buds had the same morphological value,

but he held that if, in spite of the difference in constitution of the two kinds of bud, an attempt were made to bring the one type into line with the other ("si . . . . on s'éfforçait de rattacher les bourgeons rhizogènes au type des bourgeons à rameaux"), it would be necessary to admit an analogy, physiological rather than morphological, between the rhizogenous buds and the basal internode of the branch buds, and to suppose that in the rhizogenous buds the vegetative apex was completely abortive. JANCZEWSKI further noted that if the rhizogenous bud of *E. limosum* produced two roots, these were inserted on opposite sides of the branch, one toward and one away from the organic apex of the main branch. Where there were four such roots they formed an irregular quadrangle. From the state of development of the roots JANCZEWSKI concluded that the first root to develop was that on the lower side of the branch, and the second that opposite to this, the roots between them being of later origin. Nevertheless, both his figures of young rhizogenous buds show a pair of very young roots in essentially the same stage of development.

More recently, JEFFREY (5), in treating of the rhizome of *Equisetum*, also distinguished between nodal buds which develop as rhizophoric organs and the less frequent ramular buds. I doubt whether the distinction drawn by these investigators is a valid one. For instance, JEFFREY, treating of the rhizome of *E. silvaticum*, states that in the section of it figured by him the central cylinder gives off six processes, five of which are root bases and one a dormant branch. He further points out that on one side of this section the leaf traces have entirely cleared the central cylinder, and an examination of his photograph of the rhizome shows that it is between the two traces that are farthest out that his branch bud is found. It is certainly suggestive that in serial sections through the node of the rhizome of *E. giganteum* the three branches had, at a certain level, much the appearance of JEFFREY'S root bases or rhizophoric organs, while slightly higher up they resembled his branch buds. If JEFFREY'S section, instead of being itself slightly oblique or of passing through a slightly oblique node, had passed through a region in which the traces in the upper part of the photograph were cut as far out in the cortex as those in its lower part, the rhizogenous buds

might have shown many of the same features as the branch buds. A correct conception of the form of the dormant rhizogenous buds can only be obtained from the study of serial sections, the thickness and orientation of which are known with accuracy. Such sections can hardly be obtained except with a microtome. In *E. giganteum* the dormant branch turns sharply upward toward the organic apex of the main rhizome, immediately above the first ramular dia-

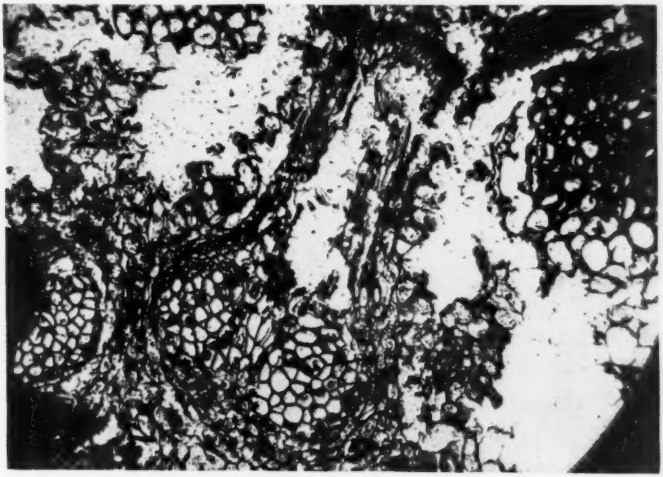

FIG. 2.—Transverse section cut slightly higher than fig. 1, showing one of bundles just reconstituted, and at periphery the insertion of two equivalent root steles at level of first diaphragm of branch; note occasional presence among tracheids of elements with lumen partially or completely obliterated; ×50.

phragm. Also, in transverse sections of the main axis, which show a radial longitudinal view of the ramular stele at its insertion on the central cylinder of the parent axis, the branch appears to terminate bluntly with the first diaphragm (fig. 2). At or just below this the root steles may be seen departing from opposite sides of the ramular stele. Only the study of the following sections reveals the existence of a distinct vegetative apex, with, slightly higher up, indications of the ochreola (fig. 3). JEFFREY'S processes given off by the central cylinder may well represent the outer, lower edges of the obliquely inserted ramular siphonostele which they closely resemble. Simi-

larly, JANCZEWSKI's two figures of very young stages of rhizogenous buds of *E. limosum* show a stage which is much like what the similar stage, that before the development of vascular tissue, must have been in the root bearing branches of the node of the rhizome of *E. giganteum*. In fig. 2 of JANCZEWSKI's pl. II in particular, the initiation of the roots can clearly be seen, while the branch appears to terminate without a vegetative apex, just as it does in the more

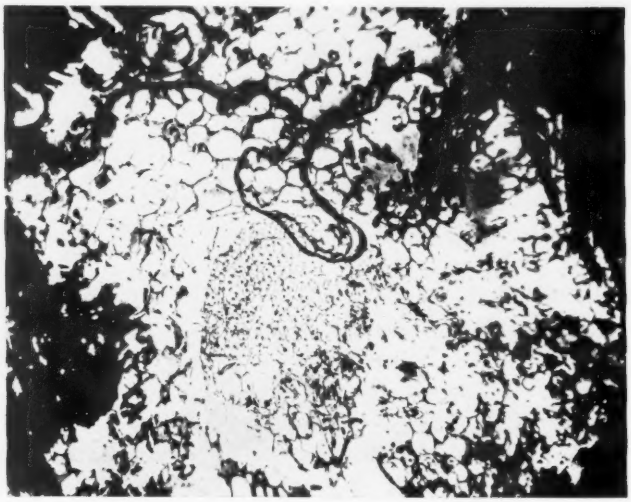

FIG. 3.—Oblique section of apex of branch above level of figs. 1 and 2; note young ochreola; toward periphery is seen part of sclerized sheath of root bearing branches; ×56.

advanced rhizogenous branches of *E. giganteum*, the apices of which lie in a higher plane. The same thing applies to the diagrammatic figure of a more advanced branch shown in JANCZEWSKI's fig. 5 of pl. II. In this connection it is significant that, although JANCZEWSKI stated that as a rule the mother cell of a rhizogenous branch does not become or give rise to an apical cell, he added that the arrangement of the cells in the young bud was sometimes such that the pre-existence of such a growing point might be presumed. He held that in such cases the apical cell early underwent extinction by dividing up

in the same way as its first segments. Although the analogy with the rhizogenous buds of *E. giganteum* suggests that a growing point may well be present in the similar organs of *E. limosum*, an abortion of this growing point in certain rhizogenous branches is not impossible of course. JEFFREY'S researches show that a single dormant branch of *E. limosum* may give rise to as many as six roots, and it is not improbable that with greater root development the axial part of the branch might become further reduced, and its apex abortive.

Increase of bundles in rhizome

As already stated, the lowest portion of the rhizome preserved is internodal, and contains nine bundles. The upper internode, although of much the same diameter, contains eleven bundles. One of these fresh bundles was formed by the breaking of the nodal xylem between the breaks formed opposite to two traces that had departed. In this region the two traces were relatively far apart. There was over the whole circumference of the stele a tendency for the traces to arise at slightly unequal distances from one another, and by no means always exactly opposite to the parenchymatous bridges which they have to traverse in order to enter the sheath. It is curious that it was not the traces on either side of the rather bulky rhizogenous branches which were relatively far apart.

The other new bundle arose in the upper part of the lower internode, between two bundles that were unusually far apart. At its first origin it was quite free from either of its neighbors, although much nearer to one of them than any of the other bundles were to the others. The first indication of the new bundle is the appearance of a single large tracheid, which is obviously of the nature of metaxylem. It is separated from the bundle nearest to it by about the width of an ordinary internodal bundle, and is situated at about the depth of the outer limit of the carinal canals. A little higher up other somewhat smaller tracheids, also obviously belonging to the metaxylem, make their appearance on the side toward the nearest bundle. The new bundle is then represented by a a nearly transverse band of metaxylem. The tracheids do not at first increase rapidly and the band remains uniseriate. A little more than a millimeter above the first appearance of the large tracheid referred to

there were but five to seven tracheids. At this level the only other indications of fascicular development is the presence of a tract of parenchyma, approximately equal in size to the bundle, a tract of tissue which would normally have been destroyed by the development of a vallecular canal. A little higher up the number of tracheids begins to increase rather more rapidly, and some of them are more deeply seated, although they still obviously belong to the metaxylem. Soon there are eleven or twelve of these, as well as a single very large, more internally situated tracheid. Thus the metaxylem found at this level, although differently disposed, is about equal in amount to that present in other bundles. Somewhat higher up the new strand contains about twenty of these tracheids, and in places the metaxylem may be as much as three elements in depth. The cells surrounding these tracheids have remained smaller than the other cortical cells, but have undergone no special differentiation, so that there is neither phloem nor protoxylem in the new strand. It is only about 3 mm. above the appearance of the first tracheid that one or two cells of protophloem make their appearance. As does the metaxylem, the phloem arises first on the side of the new bundle away from the nearest neighboring bundle, and its subsequently developed elements arise on the side toward this bundle. Just before the appearance of the phloem the new strand has become inclosed in a common bundle sheath with its nearest neighbor. About 350 μ above the appearance of the first element of phloem, a very large tracheid, about three times the size of the larger of the other metaxylem tracheids, develops on one side of the new strand, in a position markedly internal to the other tracheids, from which it is separated by five or six cells. The first indications of protoxylem appear above this tracheid. The protoxylem is represented by a small canal with the remains of three or four tracheids in it. This canal lies at about the same depth as did the large tracheid just mentioned, but it is more centrally situated with reference to the new bundle as a whole. The protoxylem canal widens rather rapidly. When the protoxylem makes its appearance, the tracheids in the middle of the band or group of protoxylem are replaced by parenchyma, so that the new bundle, although smaller, is similarly constituted to the others. It remains exceptionally close to one of its neighbors. One of the

three branches found at the node is inserted partly on the new bundle and partly on its more distant neighbor. The new bundle gives rise to a normal trace, so that there are ten of these at the node.

Summary

1. In the only specimen of the rhizome of *E. giganteum* in which the internal structure has been studied, each of the bundles of the internode is surrounded by a special sheath. There is no common endodermis.

2. All the tracheids, those of the protoxylem as well as those of the metaxylem, have their walls reticulately thickened.

3. The protoxylem is discontinuous at the nodes.

4. The basal region of the ramular siphonostele, that is, that below the first diaphragm of the branch, shows a certain differentiation of the xylem. Most of the tracheids are of the usual nodal type: short, wide, and with numerous reticulations. At the inner edge of the stele, however, there are in places a few much narrower, elongated elements with a thinner reticulum. These are not continuous with the protoxylem elements of the axis, although they resemble the latter in form, and in the appearance of the reticulum on their walls.

5. The nodal wood of the rhizome is poorly developed as compared with that of the aerial stem, and there are indications that it develops late in the ontogeny.

6. Interspersed among the nodal tracheids are parenchymatous cells of the same shape as the tracheids. These retain their cell contents.

7. In some of the nodal tracheids the lumen is nearly or completely obliterated by sclerized thickening of the cell wall.

8. In the neighborhood of the node, somewhat below the departure of the traces, the carinal canals may contain a substance of an apparently mucilaginous nature.

9. The leaf traces are very minute. Their xylem usually consists of 2–4 reticulate tracheids given off by the protoxylem. In the cortex the traces become concentric.

10. The root bearing branches of the rhizome may remain undeveloped. In the specimen in question there were no indications of

vascular structure above the first diaphragm of the branch. Each branch gave rise to two roots, the steles of which were given off from exactly opposite points. The roots and their steles appeared to be of the same age.

11. All the internodal portions available for study were mature, so that it is not possible to state whether the elements of the internodal lateral strands of metaxylem developed centripetally or centrifugally.

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POLLEN MORPHOLOGY AS AN INDEX TO PLANT RELATIONSHIP

I. MORPHOLOGY OF POLLEN

MAXY ALICE POPE

(WITH PLATE VIII)

Introduction

In the summer of 1922 the writer began to devise a key whereby pollen of the common native flowers of northern Colorado could be identified microscopically. As the work progressed, it broadened out and much information accumulated, both as to native and introduced species. This material was arranged by families and formed an extended account, from which the present paper has been extracted. It is hoped that this larger systematic account may be published at some later time.

A grant from the National Research Council made it possible to undertake this work. My thanks are due to DR. FRANK E. LUTZ for suggesting the study, and also to Professor T. D. A. COCKERELL for material which he has furnished. To Professor FRANCIS RAMALEY I am under special obligations for his continued interest and valuable suggestions during the progress of the work.

Very little literature was found pertaining to the morphology of pollen. KERNER and OLIVER[1] give a brief account of the subject. A book by EDGEWORTH,[2] published in 1877, has about 500 illustrations, but unfortunately it is impossible to determine which figures belong to a particular description. ENGLER and PRANTL[3] figure pollen of many families of plants. The most recent publication dealing with the subject of pollen is a book by SCHEPPEGRELL,[4] which contains many microphotographs of the more common hayfever producing plants. Nearly all textbooks of botany have some figures and brief description of pollen.

[1] KERNER and OLIVER, The natural history of plants. Vol. II. New York. 1895.

[2] EDGEWORTH, M. P., Pollen. London. 1877.

[3] ENGLER, A., and PRANTL, K., Natürlichen Pflanzenfamilien. Berlin. 1897.

[4] SCHEPPEGRELL, WILLIAM, Hayfever and asthma. 1922.

Methods

Many methods for the preservation of pollen grains were tried. Most of them proved unsatisfactory, presumably because of the differences in osmotic pressure of preservative and pollen grain. The following method was finally adopted. The pollen was shaken from the mature stamen upon a clean slide. A clean cover-glass was ringed with balsam, and the grains mounted dry. Balsam mounts also were usually made of each pollen. In no case was the shrinkage of any pollen noted either in the dry mounts or in balsam. Perhaps the most common artefact is the swelling of ellipsoidal, pyramidal, and polyhedral grains in balsam, producing in such cases a spherical shape. Hence for correct determinations the balsam mounts are practically worthless, unless the grain was originally of the spherical type. Balsam mounts do have a certain value, however, in that in them it is possible to distinguish any peculiar nuclear structures or differences in the relative thickness of the coats.

Investigation

Grains separate and in sacs

The great majority of flowering plants produce their pollen grains in large quantities, but each grain is separate from every other. Such is the condition in Poaceae, Ranunculaceae, Rosaceae, Fabaceae, Polemoniaceae, Scrophulariaceae, Compositae, and many other families. There are exceptions, however, some families producing grains in definite sacs, as the Orchidaceae and Asclepiadaceae, two widely separated families. One of the families studied, Onagraceae, has grains held together by viscid protoplasmic threads. All of its members thus far examined have this marked characteristic. These grains are well characterized by their shape; they are pyramidal, and the threads appear to be attached to each of the apices of the grain. In the Ericaceae the mature pollen grains occur not singly, but in tetrads. In the four families just mentioned there are relatively few grains produced per flower. The special devices which they possess are valuable, in that there is an increased assurance of the grains being carried from one plant to another by insect visitors, and since fewer grains come to nothing, fewer need be produced.

Dryness or stickiness

Pollen grains vary not only in shape, size, color, etc., but also in being either very dry and powdery or quite sticky in nature. This latter character seems to be correlated with the amount of nectar secreted by the plant. Those plants which produce large amounts of nectar have pollen grains which also seem to exude a sticky or sugary fluid. This is noticeable when one is making dry mounts of pollen, because the grains tend to remain in masses, held so by this sugary secretion. This condition, of course, should not be confused with that in the Orchidaceae, Onagraceae, or Asclepiadaceae. Some of the well known families producing sticky pollen grains are Iridaceae, Rosaceae, Fabaceae, and Compositae. Plants of these families for the most part are insect pollinated, so that the sticky pollen is an advantage.

Numerous families produce dry powdery pollen. In such cases the quantity greatly exceeds that in families in which the pollen is more or less sticky. Thus the Pinaceae, Poaceae, Salicaceae, Betulaceae, Zygophyllaceae, Chenopodiaceae, Ambrosiaceae, and others usually produce enormous quantities of dry powdery pollen. It is evidently advantageous for wind dispersal to have the grains light and dry.

Shape

One of the best characters for use in a key seems to be that of shape. All of the pollens thus far examined fall into some half-dozen groups. The great majority of the families studied have pollen grains ellipsoidal or else cylindrical (capsule-shaped), as, for instance, Nymphaeaceae, Brassicaceae (fig. 13), Capparidaceae (fig. 8), Rosaceae, Fabaceae (fig. 15), Linaceae, Loasaceae, Ammiaceae, Gentianaceae, Hydrophyllaceae, Lamiaceae, Scrophulariaceae, Compositae, etc. The group of perhaps next consequence has spherical pollen grains, including Chenopodiaceae, Nyctaginaceae, Zygophyllaceae, Malvaceae (fig. 1), Papaveraceae, Campanulaceae, Ambrosiaceae (fig. 4), etc. In the third group are Poaceae with grains in the form of a truncated pyramid or frustrum of a pyramid (fig. 16), and Betulaceae, Alsinaceae, Grossulariaceae (fig. 18), Cactaceae, Cichoriaceae, etc., with polyhedric grains. The fourth group is very small, containing Cyperaceae, Juncaceae, Primulaceae (fig. 17),

Santalaceae, and Onagraceae (fig. 11), with pyramidal pollen grains. A final group includes a single family of those thus far studied, namely, Boraginaceae (fig. 14), with very small grains dumb-bell shaped.

The shape seems fairly constant for all of the pollens of any one family, excepting in a comparatively few cases. For example, the pollen grains of *Convolvulus americanus* and *C. interior* are spherical, whereas those of *C. arvensis* are ellipsoidal. All of the genera of Solanaceae thus far examined have ellipsoidal pollen grains, except *Salpiglossis*, which has grains made up of four united globes. In the Caprifoliaceae, *Lonicera* and *Linnaea* have spherical grains, while *Viburnum* has ellipsoidal grains; *Catalpa speciosa* has large spherical grains with numerous warty knobs, but *Tecoma* has ellipsoidal grains. In the Acanthaceae, according to the figures of ENGLER and PRANTL, we find spherical, ellipsoidal, oblong, and many different types and sizes of grain.

SIZE

The size of pollen grains seems to be fairly constant in any species, and even among all of the species of a genus; often also among the genera examined in a family. This character is important in the identification of families by the key which has been worked out. In some cultivated plants there are often pollen grains of two distinct sizes, indicating perhaps a hybrid origin. This condition was noticed in the garden rose, tulip, and hothouse *Oxalis* for cultivated plants, and in *Douglasia montana* and *Sidalcea asprella* of the native plants.

The differences in size of pollen from various families is remarkable. Some grains are very large and visible to the naked eye, as is shown by the following measurements (in microns): *Zea mays* 160, *Humulus neomexicanus* 100–140, *Althaea rosea* 100, *Gossypium* 100, *Salpiglossis sinuata* 108. Other grains are so small as to require a compound microscope for detection, as in all of the Monotropaceae, Primulaceae, Apocynaceae, Santalaceae, and Boraginaceae. Examples are: *Hypopitys* 12, *Cyclamen europaeum* 16, *Apocynum* 22, *Mertensia lanceolata* 10.

COLOR

The colors of pollen grains seem to be almost constant for any one family of plants, only one or two exceptions having been noted.

The colors vary from the deep orange-yellow characteristic of the members of the Portulacaceae, Fabaceae, Compositae, and others, to the pale, almost cream colors of such families as the Caryophyllaceae, Primulaceae, Polemoniaceae, and Bignoniaceae. Some grains are more or less bluish in color, as for example those of the Onagraceae, and some of the Polemoniaceae; others are characterized by a greenish color, noticeable in *Cleome* (Capparidaceae). The great majority of plant families seem to have pollen with a more or less uniform, ordinary yellow color, as Poaceae, Betulaceae, Ranunculaceae, Brassicaceae, Rosaceae, Malvaceae, Scrophulariaceae, etc.

Color alone, therefore, is insufficient for purposes of identification, although it helps if other characters are mentioned. The color probably is important in the insect world. It seems that those grains of a deep yellow, blue, or greenish color are produced on flowers which are chiefly insect pollinated, whereas some of the more common yellow ones are scattered by other means, principally the wind, as in Poaceae and Betulaceae.

Pollen wall

As a rule, the wall of a pollen grain is three-layered. According to Kerner and Oliver, these layers are the outer or perine, the middle or extine, and the internal or intine. The intine is usually very thin, and closely attached on one side to the extine and on the other to the protoplasm. The extine varies in thickness, apparently depending upon the shape of the pollen grain, but remaining constant for each particular shape. Thus all spherical grains have an extine which is usually more than 3 μ thick, and in some cases 8–10 μ. Most of the grains having extines less than 3 μ are either polyhedric or ellipsoidal in shape. Grains with thick extines are more apt to have pores than those with a thinner middle coat. The perine always is very thin, and is deposited from the matrix in which the young pollen grain is imbedded. It is closely associated with the extine, and is hard to distinguish. Kerner and Oliver state that the various sculpturings, prickles, and other irregularities of the outer coat really appertain to the perine. In the present study the term extine includes both perine and extine.

EXTERNAL MARKINGS

The best, most constant, and readily distinguishable character by which grains may be identified is smoothness or non-smoothness of the extine, or, in other words, the external marking of the grains. The divisions under which all grains seem to fall in regard to this character are extine smooth, reticulate, punctate, echinate, with pores.

EXTINE SMOOTH.—In many families the pollen grains have no characteristic markings, the surface appearing smooth, as in Poaceae (fig. 16), Commelinaceae, Betulaceae, Grossulariaceae (fig. 18), Rhamnaceae, etc. These families are further characterized, of course, by size, shape, and color.

EXTINE RETICULATE.—A great majority of grains have thickenings of the extine, which have a more or less definite arrangement or pattern. These thickenings are usually spoken of as reticulations. In some families the patterns or facets made by the reticulations are rather small and quite regular, as Capparidaceae (fig. 8), Oxalidaceae, Zygophyllaceae, Linaceae, Caesalpinaceae, and Polemoniaceae; whereas in other families the reticulations are distinct, but more or less irregular, as in Nyctaginaceae (fig. 7), Saxifragaceae, Rosaceae, Vitaceae, Loasaceae, and Solanaceae. In some families reticulations are present together with pores, and it is not unusual to find a pore in the center of each facet.

EXTINE PUNCTATE.—The presence of pits or punctures on the pollen grain is very common, and occurs in Papaveraceae, Brassicaceae (fig. 13), Fabaceae (fig. 15), Scrophulariaceae, Lobeliaceae, etc.

EXTINE ECHINATE.—One of the most interesting of the variations manifested by pollen grains is found in the echinations or prolongations of the extine into definite spines. The number, length, and position of these spines vary in the different plant families in which they occur, and constitute some of the most exact characters for identification purposes. In some families the spines are very long and quite striking, as in all members of the Malvaceae (fig. 1) thus far examined and also in the Nymphaeaceae. All members thus far studied of Valerianaceae, Ambrosiaceae (fig. 4), and Compositae (fig. 6), however, have very much shorter and more numerous spines. Grains may also have pores along with these echinations, as in

Caprifoliaceae (*Lonicera* and *Linnaea*), Malvaceae, Campanulaceae, and Ambrosiaceae (these show only in balsam mounts).

The shape of the grain may be correlated with the presence of echinations, as all grains thus far found possessing spines are either spherical (Caprifoliaceae, Malvaceae, and Ambrosiaceae, figs. 1, 3, 4) or ellipsoidal (Nymphaeaceae, Valerianaceae, and Compositae, fig. 6). The dumb-bell, cubical, or polyhedric-shaped grains may have markings, but never prolongations of the extine into definite spines, except in the case of Cichoriaceae, in which the polyhedric grains do have spines (fig. 5).

EDGEWORTH noted echinate pollen grains in twenty-one families. The writer has found echinations in species from five of these families. These are placed first in his list which follows: Campanulaceae, Compositae, Valerianaceae, Caprifoliaceae, Nymphaeaceae, Smilaceae, Butomaceae, Taccaceae, Polygonaceae, Verbenaceae, Acanthaceae, Convolvulaceae, Ficoideae, Cactaceae, Droseraceae, Sapindaceae, Dicenaeaceae, Geraniaceae, Sterculiaceae, Berberideaceae, and Ranunculaceae. In addition to these, echinate grains have been observed in the present study in Ambrosiaceae and Malvaceae.

EXTINE WITH PORES.—Many of the families have no particular extension of the extine, but are decidedly different in that there are pores present. The size, number, and position of these pores vary with the different families. Families in which pores are conspicuous are Chenopodiaceae, Nyctaginaceae (fig. 7), Caryophyllaceae, Grossulariaceae (fig. 8), Malvaceae (fig. 1), Cactaceae, Onagraceae (fig. 11), and Campanulaceae. In some of these families the arrangement of the pores is striking, and in most cases constant. As an example may be named the Onagraceae, which so far as studied are found to have tetrahedral grains. There are four pores, one at each apex; usually only three of these are visible at one time (fig. 11). These are not complete openings in the extine, but simply thinner places in the outer covering. In Grossulariaceae (fig. 18) one large pore is present in the center of each one of the twelve faces of the grain. When the pollen tube develops it grows out through one of these pores.

INTERNAL DIFFERENTIATION

Since the writer's chief interest has been in external morphology, very little time has been spent in a study of internal differentiation.

TABLE I

CLASSIFICATION OF POLLEN GRAINS ACCORDING TO SHAPE, SIZE, AND EXTERNAL MARKINGS; S, SMALL GRAINS, (10-30 μ); M, MEDIUM GRAINS (30-60 μ); L, LARGE GRAINS (60-100 μ)

Kind of surface and markings	Elongated ellipse	More than one division	Spherical	Dumb-bell shaped	Ellipsoidal	Cylindrical (capsule-shaped)	Pyramidal	Polyhedric
Echinate			Malvaceae, L Ambrosiaceae, S Caprifoliaceae, M Campanulaceae, S		Nymphaeaceae, L Valerianaceae, L Dipsaceae, L Compositae, M Caprifoliaceae, M			Cichoriaceae, M
Reticulate	Convallariaceae, M Melanthaceae, M Amaryllidaceae, M Alliaceae, M	Pinaceae, L	Polemoniaceae, M Zygophyllaceae, M Nyctaginaceae, M Geraniaceae, L Fumariaceae, S Cannabinaceae, L Bignoniaceae, L		Cesalpiniaceae, M Capparidaceae, S Oxalidaceae, M Oleaceae, M Gentianaceae, M Solanaceae, M Menyanthaceae, L Loasaceae, M Saxifragaceae, S Euphorbiaceae, M Linaceae, L Iridaceae, L Anacardiaceae, M Cannabinaceae, L Bignoniaceae, M	Rosaceae, M Vitaceae, S	Juncaceae, M	
Smooth	Commelinaceae, M	Ericaceae, M Apocynaceae, S		Boraginaceae, S	Oleaceae, S Araceae, M Convolvulaceae, L Rhamnaceae, S	Crassulaceae, S Loganiaceae, S Ammiaceae, M Polygonaceae, M		Betulaceae, S Grossulariaceae, S Monotropaceae, S Poaceae, M
Punctate	Scrophulariaceae, M Violaceae, M Liliaceae, L		Nyctaginaceae, L Caryophyllaceae, M		Dipsaceae, L Amygdalaceae, S Araliaceae, S Scrophulariaceae, S Brassicaceae, M Lobeliaceae, M Papaveraceae, M	Vitaceae, S Fabaceae, S Hydrophyllaceae, M	Onagraceae, L Santalaceae, S Primulaceae, S Cyperaceae, S	Alsinaceae, M
Verruculose		Solanaceae, L	Geraniaceae, L				Onagraceae, L	Cactaceae, L Curcubitaceae, L Ranunculaceae, S

TABLE I—*Continued*

Bands			Acanthaceae, M			Ammiaceae, M Polygonaceae, M	Juncaceae, M	
Furrows or grooves	Scrophulariaceae, M Violaceae, M Liliaceae, L Convallariaceae, M Melanthaceae, M Amaryllidaceae, M Alliaceae, M		Fumariaceae, S		Oleaceae, M, S Gentianaceae, M Solanaceae, M Menyanthaceae, L Loasaceae, M Saxifragaceae, S Papaveraceae, M Lamiaceae, S Amygdalaceae, S Araliaceae, S Scrophulariaceae, S Brassicaceae, M Lobeliaceae, M Capparidaceae, S Oxalidaceae, M Euphorbiaceae, M Linaceae, L Iridaceae, L Anacardiaceae, L Compositae, M Convolvulaceae, L	Vitaceae, S Fabaceae, S Hydrophyllaceae, M Crassulaceae, S Loganiaceae, S	Santalaceae, S Primulaceae, S	
With distinct pores			Malvaceae, L Ambrosiaceae, S Caprifoliaceae, M Portulacaceae, M Convolvulaceae, L Papaveraceae, M Geraniaceae, L Carophyllaceae, M Amaranthaceae, S Chenopodiaceae, S Campanulaceae, S		Valerianaceae, L		Onagraceae, L	Grossulariaceae, S Ranunculaceae, S Alsimaceae, M Curcubitaceae, L Cactaceae, L

A few facts have been noted, however, especially in regard to the nucleus, which seems in all cases to be central in position. The nucleus is quite easily seen in stained material, and if unstained material is properly examined can be distinguished with proper focusing. When unstained the nucleus appears darker and denser and in all ways like the nuclear structure of any typical plant cell. In regard to cytoplasmic structure, it seems, in the main, to resemble any young plant cell in which the entire space between nuclear membrane and cell wall is filled with a more or less homogeneous, alveolar material.

TABULATION OF DIFFERENCES

Table I gives in condensed form much of the information obtained from examination of pollen grains of plants available for study. As will be noted, the same family may occur in more than one place in the table, because of differences in grains within the family.

Summary

The paper reports a study of pollen morphology in numerous genera of about eighty families represented in the flora of Colorado, together with a small number of familiar exotic forms. A discussion is given of the general interest of pollen, with methods of study and preservation. Pollen shapes are described, as well as markings, size, color, stickiness, abundance, and other features.

It is found that, in general, the shapes and sizes of grains in the various genera of a family correspond rather closely, but there are some families which show striking differences. The most common shape of pollen grains among dicotyledons is short ellipsoidal. Most of the higher as well as many of the lower families have such ellipsoidal pollen. Other common shapes are spherical, pyramidal, polyhedral, etc. Among the monocotyledons, many families have grains elongated ellipsoidal. The same shape occurs also among some of the dicotyledons. In the Poaceae the grains are pyramidal or like a frustrum of a pyramid.

As to external features, it has been found possible to classify grains as echinate, reticulate, smooth, punctate, verruculose, etc. It is a conspicuous fact that many of the higher families of dicotyledons have echinate pollen.

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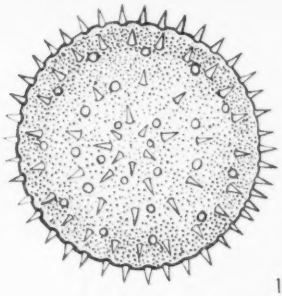

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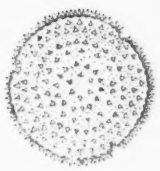

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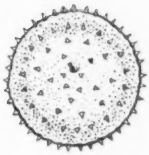

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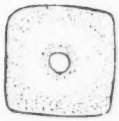

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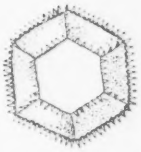

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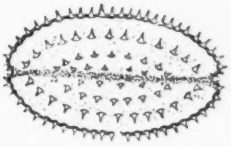

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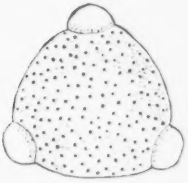

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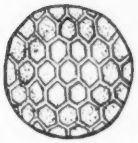

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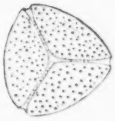

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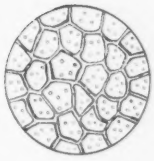

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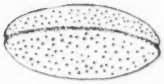

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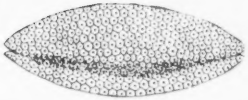

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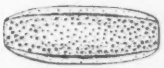

13

POPE on POLLEN MORPHOLOGY

EXPLANATION OF PLATE VIII

The figures were drawn with Abbe camera lucida, Bausch and Lomb 4 mm. objective and ocular 10. They were made from pollen grains mounted dry. The magnification of the figs. 1, 3, 5, 7, 9, 11, and 15 was 625; of figs. 2, 4, 6, 8, 10, 12, 13, 16, 17, and 18 *a*, *b*, *c*, 1250; of fig. 14, 1875. They are reduced approximately one-third in the process of reproduction.

FIG. 1.—*Sidalcea asprella* Greene (Malvaceae); large spherical echinate pollen grain; ×312.

FIG. 2.—*Monarda* sp.? (Lamiaceae); small ellipsoidal pollen grain with reticulations; ×625.

FIG. 3.—*Lonicera sempervirens* L. (Caprifoliaceae); medium sized spherical pollen grain with short echinations; ×312.

FIG. 4.—*Ambrosia elatior* L. (Ambrosiaceae); small spherical echinate pollen grain with pores; ×625.

FIG. 5.—*Nothocalais cuspidata* (Pursh) Greene (Cichoriaceae); medium sized polyhedral echinate pollen grain; ×312.

FIG. 6.—*Grindelia oregana* A. Gray (Compositae); medium sized or small ellipsoidal pollen grain with spines and pores; ×625.

FIG. 7.—*Abronia fragrans* Nutt. (Nyctaginaceae); medium sized pollen grain with reticulations; ×312.

FIG. 8.—*Peritoma angustum* (M. E. Jones) Rydb. (Capparidaceae); small ellipsoidal pollen grain with grooves and furrows; ×625.

FIG. 9.—*Tribulus terrestris* L. (Zygophyllaceae); medium sized spherical pollen grain with regular reticulations; ×312.

FIG. 10.—*Toxicoscordion falcatum* Rydb. (Melanthaceae); medium sized pollen grain, shape of an elongated ellipse, and having reticulated surface; ×625.

FIG. 11.—*Chamaenerion spicatum* (Lam.) S. F. Gray (Onagraceae); large tetrahedral pollen grain with large pores at corners; ×312.

FIG. 12.—*Capnoides montanum* (Engelm.) Britton (Fumariaceae); small spherical pollen grain with grooves; ×625.

FIG. 13.—*Draba streptocarpa* A. Gray (Brassicaceae); medium sized pollen grain with grooves; ×625.

FIG. 14.—*Mertensia brachyloba* Greene (Boraginaceae); small dumb-bell shaped pollen grain; ×037.

FIG. 15.—*Petalostemon purpureus* (Vent.) Rydb. (Fabaceae); small capsule-shaped pollen grain with punctate surface; ×312.

FIG. 16.—*Bromus Porteri* (Coult.) Nash (Poaceae); medium sized pollen grain shape of frustrum of pyramid; ×625.

FIG. 17.—*Primula Parryi* A. Gray (Primulaceae); small pyramidal pollen grain with punctate surface; ×625.

FIG. 18.—*Ribes longifolium* Nutt. (Grossulariaceae): *a*, side view; *b*, view of lateral face; *c*, face view of base; small polyhedric pollen grain with smooth surface and distinct pores; ×625.

GROWTH OF WHEAT ROOTS IN SALT SOLUTIONS CONTAINING ESSENTIAL IONS[1]

SAM F. TRELEASE AND HELEN M. TRELEASE

(WITH TWO FIGURES)

Retardation of root growth has been used in many studies as an index of the injurious or toxic action of various chemical agents upon plant protoplasm. Roots are specially suitable for such studies, because their protoplasm is readily accessible to the toxic agent. The growth responses of roots immersed in solutions containing the ions that are essential for plant growth have received relatively little attention. Since the roots are the organs through which all of the salts usually enter the plant, the effects of nutrient salts upon root growth are of special physiological importance. These considerations have led the writers to an attempt to secure some quantitative data on the rate of elongation of wheat roots in aqueous solutions containing nutrient salts. The results indicate some of the relations that exist between the composition of the culture solutions and the growth made by the roots, and furnish information concerning the salt proportions that are favorable for root development.

Method

The thirty-seven culture solutions used contained the three salts, potassium dihydrogen phosphate (KH_2PO_4), calcium nitrate ($Ca(NO_3)_2$), and magnesium sulphate ($MgSO_4$), in various sets of proportions. Each solution had a total volume-molecular concentration of 0.06 gram-molecule per liter, but the solutions differed in the volume-molecular proportions of the component salts. The volume-molecular proportions of the salts are given in table I, and the compositions of the solutions are represented graphically by means of points shown on the equilateral triangle of fig. 1. Near the points are numbers corresponding to the solution numbers in table I. The upper apex of the diagram represents a solution in which 100

[1] Botanical contribution from the Johns Hopkins University.

per cent of the dissolved molecules are KH_2PO_4; the left apex, one in which 100 per cent of these are $MgSO_4$; and the right apex, one in which 100 per cent of the solute molecules are $Ca(NO_3)_2$. Points on the base of the triangle denote various molecular proportions of $Ca(NO_3)_2$ and $MgSO_4$, the percentage of KH_2PO_4 being zero. Points on the left side represent solutions containing various molecular proportions of KH_2PO_4 and $MgSO_4$, these solutions containing no $Ca(NO_3)_2$. Points on the right side denote solutions containing $Ca(NO_3)_2$ and KH_2PO_4, but no $MgSO_4$. Points in the interior of the triangle represent solutions that contain all three salts.

Spring wheat of a pure line was employed in these tests (Marquis, Saskatchewan, no. 70, Selection no. 313, supplied by the University of Saskatchewan, through the kindness of Professor MANLEY CHAMPLIN). After soaking for three hours in tap water, the seeds were sprouted on wet blotting paper in a moist chamber. When the first root of each seedling was about 4 mm. long, the seedlings were transferred to the culture vessels, which were ordinary glass tumblers with a capacity of about 275 cc. A piece of paraffined bobbinet (somewhat like mosquito netting, but with hexagonal meshes and firmly fixed threads) was stretched over the top of the tumbler and fastened by a ligature of paraffined linen thread. The tumbler stood in a 600 cc. beaker (Griffin low form), and both the tumbler and the space around it were filled with the solution, the level of the latter being even with the top of the tumbler in both cases.

Fifty seedlings were used in each culture. They were placed upon the netting at the surface of the solution, care being taken that every root dipped into the solution and that the seeds were not flooded. The cultures were kept in darkness, and a moist chamber was provided for each during the first two days by covering the beaker with an inverted watch glass (10.5 cm. in diameter). When the roots in solution 23 (which has equal molecular proportions of the three salts) had attained a length of about 70 mm., all the plantlets of cultures in the series were harvested, and the length of the longest root on each seedling was recorded.

These experiments were begun in May 1923, at the Laboratory of Plant Physiology of the Johns Hopkins University, and they were continued at the University of Louisville. In the different series the

temperature ranged from 18° to 23° C., and the corresponding time required for the roots in the standard solution to attain a length of about 70 mm. ranged from 89 hours to 69 hours. The writers are pleased to acknowledge indebtedness to Professor B. E. LIVINGSTON for suggestions in the preparation of this paper.

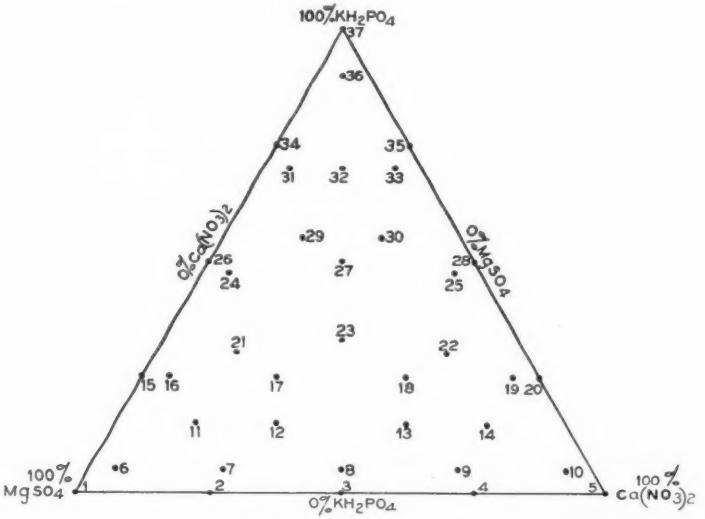

FIG. 1.—Triangular diagram showing culture numbers and volume-molecular salt proportions of one-, two-, and three-salt solutions.

Results

The results of several series are summarized in table I. In each series from twelve to sixteen cultures usually were employed, and the relative amounts of growth made in the various solutions always were expressed as percentages of the corresponding growth in solution 23. The value given for each individual test (fifth column of the table) is based upon a mean for fifty seedlings. The average for each solution is the mean of all individual values for that solution. The relative physiological values of the various solutions were derived by expressing each value in the preceding column as a percentage of the highest value in that column.

To bring out relations between root growth and the molecular

proportions of the salts employed, relative physiological values are shown on the triangular diagram of fig. 2, which is similar to fig. 1 except that the physiological values replace the culture numbers. The contour lines on the diagram have been drawn so as to divide the area of the triangle into seven zones, representing the following

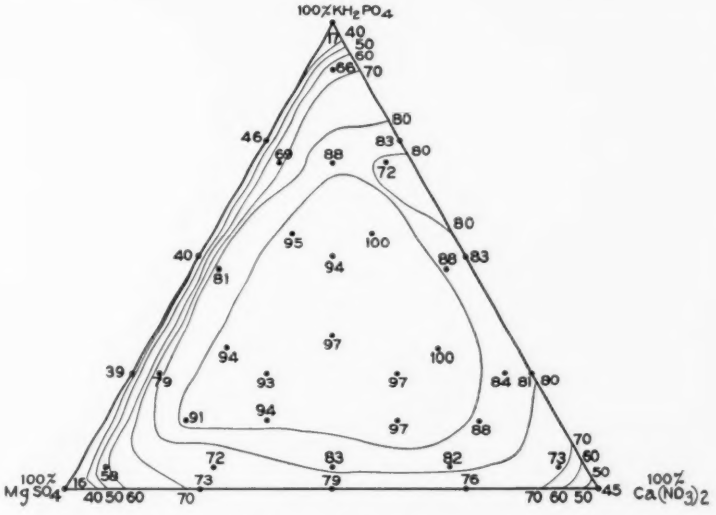

FIG. 2.—Relative physiological values of solutions; contour lines divide triangular area into zones of physiological values as related to salt proportions.

ranges of physiological values: 90–100, 80–90, 70–80, 60–70, 50–60, 40–50, and below 40.

The zoning brought out by these contours is very regular and consistent, the only noticeable break in the regular concentricity of the zones being that necessitated by the value shown for solution 33. The eccentricity suggested at that point on the diagram may well be significant, or it may be related only to the group of unknown experimental features connoted by the term experimental error. At any rate, the data offer no means for detailed study of the slight eccentricity in question. Considering the limitations of such experimentation as this, the zoning appears to be as consistent as might be hoped for.

The central zone indicates high relative physiological values (from 90 to 100) for a group of eleven solutions, nos. 11, 12, 13, 17, 18, 21, 22, 23, 27, 29, and 30. Any point within this zone may be expected to represent a three-salt solution with a physiological

TABLE I

SALT PROPORTIONS OF CULTURE SOLUTIONS AND CORRESPONDING RELATIVE AMOUNTS OF GROWTH MADE BY ROOTS OF WHEAT SEEDLINGS

SOLUTION NO.	RELATIVE VOLUME-MOLECULAR PROPORTIONS OF SALTS			LENGTH OF LONGEST ROOT AS PERCENTAGE OF LENGTH FOR CONTROL CULTURE IN EACH CASE*		RELATIVE PHYSIOLOGICAL VALUE OF SOLUTION
	KH_2PO_4	$Ca(NO_3)_2$	$MgSO_4$	Individual tests	Average	
1	0.0	0.0	100.0	15, 17	16	16
2	0.0	25.0	75.0	71, 79	75	73
3	0.0	50.0	50.0	78, 83	81	79
4	0.0	75.0	25.0	75, 81	78	76
5	0.0	100.0	00.0	44, 47	46	45
6	5.0	5.0	90.0	55, 60, 59, 58, 59, 65, 63	60	58
7	5.0	25.0	70.0	71, 84, 68	74	72
8	5.0	47.5	47.5	80, 89, 85	85	83
9	5.0	70.0	25.0	81, 82, 90	84	82
10	5.0	90.0	5.0	65, 72, 75, 78, 78, 83	75	73
11	15.0	15.0	70.0	94, 93	94	91
12	15.0	30.0	55.0	95, 98	97	94
13	15.0	55.0	30.0	100, 100	100	97
14	15.0	70.0	15.0	89, 93	91	88
15	25.0	0.0	75.0	38, 41	40	39
16	25.0	5.0	70.0	76, 89, 78	81	79
17	25.0	25.0	50.0	90, 97, 94, 102, 99	96	93
18	25.0	50.0	25.0	95, 103, 99, 101, 103	100	97
19	25.0	70.0	5.0	86, 85, 86	86	84
20	25.0	75.0	0.0	82, 83	83	81
21	30.0	15.0	55.0	96, 98	97	94
22	30.0	55.0	15.0	101, 104	103	100
23	33.3	33.3	33.3	100 (control)†	100	97
24	47.5	5.0	47.5	84, 81, 83	83	81
25	47.5	47.5	5.0	90, 92, 90	91	88
26	50.0	0.0	50.0	38, 43	41	40
27	50.0	25.0	25.0	93, 98, 93, 99, 103	97	94
28	50.0	50.0	0.0	82, 87	85	83
29	55.0	15.0	30.0	96, 99	98	95
30	55.0	30.0	15.0	97, 108	103	100
31	70.0	5.0	25.0	69, 72, 72	71	69
32	70.0	15.0	15.0	92, 89	91	88
33	70.0	25.0	5.0	82, 72, 68	74	72
34	75.0	0.0	25.0	43, 51	47	46
35	75.0	25.0	0.0	83, 86	85	83
36	90.0	5.0	5.0	65, 58, 72, 74, 73, 67	68	66
37	100.0	0.0	0.0	19, 17	18	17

* Duration of each experiment was the time period (approximately 88 hours at 18.5° C.) required for roots in the control solution (no. 23) to increase in length from 4 mm. to 70 mm.

† Length of longest root in control culture was taken as 100 in each case.

value of 90 or above (on the criteria here used). Furthermore, solutions 14, 25, and 32 gave physiological values of 88, only slightly lower than the lowest ones included in the central zone on the diagram, and the points for these lie close to the central zone. These three solutions may be added to the eleven preceding, and thus secure an optimum group of fourteen solutions, every one of which gave a physiological value of 88 or above.

The central area defined by these fourteen points may be examined with reference to the sets of salt proportions that are included. These solutions generally are characterized by having the partial molecular concentration of every salt as great as or greater than 15 per cent of the total molecular concentration. There is but one exception, solution 25, in which the partial concentration of magnesium sulphate is only 5 per cent of the total concentration. On account of this exception, it is probably unsafe to predict that physiological values lower than 88 may be expected for all solutions in which the partial molecular concentration of any salt is less than 15 per cent of the total molecular concentration. The generalization seems to be well indicated, however, that all three-salt solutions of this type and total molecular concentration may be expected to have physiological values of 88 or above (with the non-solution conditions and by the criteria here used), provided the partial molecular concentration of no salt is lower than 15 per cent of the total molecular concentration.

It is of interest to consider whether the data for the optimum group of solutions are adequate for an attempt at further subdivision of this group. The central area of the diagram might be subdivided by additional contour lines representing values between 90 and 100, but such subdivisions surely would be transgressing the logical limits set by the degree of precision attained in the tests themselves. The data suggest that the very best solutions may be represented by points lying to the right of the vertical diameter of the triangle, and there seems to be indication that the solution values become progressively lower toward the left corner of the optimum area. The central area must represent the surface of a plateau with definite curvatures. It is apparent from the data that no three-salt solution of this type, with a total concentration of 0.06 gram-molecule per

liter, if tested with these seeds and these non-solution conditions, would be apt to give markedly more rapid root elongation than actually was observed for solutions 22 and 30. The generalization, however, seems to be about as precise as should be attempted at present.

The molecular ratios were calculated for the eleven tested solutions of the central area. The values of the ratio of calcium nitrate to magnesium sulphate range from 0.21 to 3.67, those of the ratio of potassium dihydrogen phosphate to magnesium sulphate have the same range, and those of the ratio of calcium nitrate to potassium dihydrogen phosphate lie between 0.27 and 3.67. These results lead to the same conclusion as has been reached from experiments with longer culture periods and for later developmental phases, by GILE (1), TOTTINGHAM (3), SHIVE (2), TRELEASE (4), and others. The specific effect of any molecular ratio between two salts in a culture solution surely depends upon the complex balance of the partial concentrations of all three salts and their resulting ions. The influence of any pair of salts must be considered as determined by the relation of these salt molecules to those of the third salt, and doubtless to all other influential conditions. Theoretically, it should be possible to state an optimum range for the ratio between any two salts or between any two ions when the rest of the environmental complex is sufficiently restricted. If the rest of the complex is sufficiently altered, however, a corresponding change in the optimum range is to be expected.

If the solutions represented by the right-hand half of the optimum area really are to be considered as better than those represented by the left-hand half, then it is suggested that this feature is related to the ratio of calcium nitrate to magnesium sulphate, within the limits of the optimum area. The data do not warrant any discussion of this suggestion, however.

The relative physiological values (16 and 17) given for the simple solutions of magnesium sulphate and potassium dihydrogen phosphate (1 and 37) were the lowest of the series, but the solution (5) containing only calcium nitrate has a much higher value (45). These three determinations, which are represented by the three apices of the triangle in fig. 2, constitute a simple study of the toxic-

ity of the three salts in question, when employed with a concentration of 0.06 gram-molecule per liter. It appears that, for initial root growth under the conditions of these tests, the first two salts were about twice as effective in retarding growth as was the third.

The series includes tests of three combinations of each of the pairs of salts, the nine two-salt solutions represented on the margins of the diagram of fig. 2. The results of these tests furnish evidence of what has been called antagonism, but no analyses of this can be made from the data of only three combinations of each pair of salts. It is interesting to note that the physiological values of the three combinations of calcium nitrate and potassium dihydrogen phosphate, and the three of calcium nitrate and magnesium sulphate are all about the same (from 73 to 83), while the values for the three combinations of magnesium sulphate and potassium dihydrogen phosphate are much lower (39 to 46). These general relations might be expected from the relative toxicities of the single-salt solutions. Combinations of the two very toxic salts give much greater growth retardation than do those of either one of the very toxic salts with the slightly toxic one.

One of the conspicuous features of the zonation shown in fig. 2 is the excessive crowding of the contours near the left margin, which represents a marked fall in physiological values in passing from solutions with 5 per cent of their total molecular concentration due to calcium nitrate to those that contain none of that salt.

In the present paper no discussion is attempted of zonation, etc., with reference to the many theories of physiological and physical chemistry that might be applied to these results. These questions may be left to some future time, after there is sufficient evidence that the results themselves are reliable enough to warrant such discussion.

It should be mentioned that with other experimental technique, other solution volumes or rates of renewal, other total concentrations, other phases of development, other kinds of plants, other temperatures, etc., the comparative values obtained might have been very different from those secured in these studies. TRELEASE and LIVINGSTON (**5**) have emphasized the important influence that climatic conditions may exert in experiments with solution cultures.

In conclusion, attention may be called especially to the high degree of consistency shown by these results. Many of the inconsistencies so frequently encountered in the study of experimental results with solution cultures unquestionably are due to the extreme complexity of both the internal and the external complexes of influential conditions. A study of the initial behavior of germinating seeds may be expected to involve less complexity, within as well as outside the organism, than would be involved in a study of later phases of growth. Notably, the experiment period could be very short in the experiments here described, thus practically avoiding many of the alterations in organisms and solution that increase with time. Also, the whole question of photic environment was avoided, since such tests can be carried on in darkness. It appears that this general type of experimentation is very promising, and that really reliable solution-culture results (in the sense of physics and chemistry) may be secured if attention is confined to the first few days of seed germination. Our greatest present need in biological experimentation is that plans be worked out which will allow problems to be attacked in their very simplest form. Perhaps the main use of this paper may be that it contributes a little in that direction.

Summary

1. This paper reports a study of root growth in very young wheat seedlings supplied with solutions containing one or more of the salts potassium dihydrogen phosphate, calcium nitrate, and magnesium sulphate.

2. Thirty-seven different solutions were tested, each with a total concentration of 0.06 gram-molecule per liter. Besides the 3 single-salt solutions, the series included 9 two-salt solutions and 25 three-salt solutions.

3. Marked retardation of root elongation did not occur unless the volume-molecular concentration of at least one of the three salts constituted less than about 15 per cent of the total volume-molecular concentration of the solution.

4. The roots were not very sensitive to small differences in salt proportions, except when the partial concentration of calcium nitrate in the solution was below about 5 per cent of the total concentration.

5. The results are presented by means of triangular co-ordinates, and they are very consistent.

6. It appears that root elongation in very young seedlings furnishes a subject that is not too complex for experimentation aiming toward reliable results.

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COTYLEDON FORM AND SIZE IN RECIPROCAL HYBRIDS BETWEEN SPECIES OF DIGITALIS[1]

J. Ben Hill

(WITH FOUR FIGURES)

Seedlings of the species of *Digitalis* are characterized by two general types of cotyledons. The type characteristic of *D. purpurea* and *D. Thapsi* is small and short, distinctly broader than long; the other type of cotyledon, characteristic of *D. ambigua*, *D. lutea*, and others, is larger and in general slightly longer than broad.

During several years' study of species hybrids of *Digitalis*,[2] in crosses between various species having these distinct types of cotyledons, I have noted that the reciprocal F_1 hybrids differ considerably in the form and size of their cotyledons, and that they always resemble the female or seed parent in these respects. In fact, a resemblance to the seed parent, although difficult to describe, is apparent even in hybrids between species having the same type of cotyledon. In hybrids between those species differing in cotyledon form and size the difference is striking. The only reference found in the literature concerning a matroclinous tendency in the cotyledon characters of hybrids is that of Focke,[3] in his discussion of *Nymphaea* species hybrids, which he says have cotyledons resembling the female parent.

Investigation

Reciprocal hybrids between *Digitalis purpurea* and *D. lutea* were secured in abundance in 1923. These are distinct species, differ-

[1] Published by permission of the Director of the Agricultural Experiment Station as a part of Project no. 657. Contribution from the Department of Botany, The Pennsylvania State College, no. 50.

[2] I wish to acknowledge my indebtedness to the Botanic Gardens of Kew, Dublin, Edinburgh, and Cambridge; the Museum d'Histoire Naturelle, Paris; and especially the Jardin Botanico, Madrid, for the collections of seeds of *Digitalis* species which have been used in the study of the hybrids.

[3] Focke, W. O., Die Pflanzenmischlinge. Berlin. 1881.

ing markedly in the form and size of the cotyledons, and their hybrids furnished excellent material for the study. The customary technical precautions were observed in making the hybridizations and in growing the seedlings. Seedlings of the reciprocal F_1 hybrids and of both parents were grown. Full maturity of the cotyledons of *Digitalis* seedlings is reached within three or four weeks from planting. The observations were made at intervals of about a week, and a series of stages in the development of the cotyledons was secured and studied. Notes concerning the general appearance of the seedlings were made, and sketches to illustrate their resemblances and differences. The drawings, of which the accompanying figures show

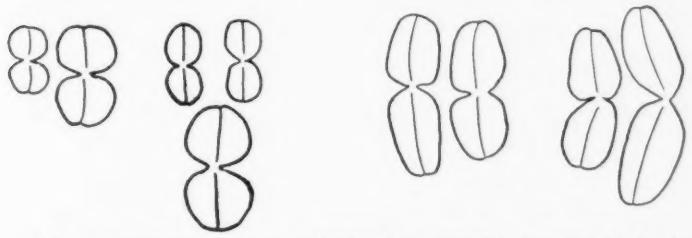

FIG. 1.—Very young stages of cotyledons about one week from date of planting; parent species at either side and reciprocal hybrids between; reading from left to right: *D. purpurea*, *D. purpurea*×*D. lutea*, *D. lutea*×*D. purpurea*, and *D. lutea*.

appropriate reductions, were camera lucida sketches showing a uniform magnification of ten diameters.

The cotyledons of *D. purpurea* are orbicular in the young stages, with the transverse and longitudinal axes about equal (fig. 1). As the cotyledons reach maturity, the transverse axis becomes greater than the longitudinal axis (figs. 3, 4). Based on an average of twenty measurements, the ratio of width to length was found to be 1.26 mm. to 1.21 mm. for the young stages. Based on an average of thirty measurements of mature cotyledons, the ratio of width to length was found to be 5.25 mm. to 4.016 mm. These ratios, expressed in percentages, are 1.045 and 1.306 respectively. The tips of the cotyledons are often retuse. The cotyledons of *D. lutea* are elliptic in the young stages (fig. 1), and become broadly ovate in the older stages (figs. 3, 4). The transverse axis is shorter than the longitudinal axis in all stages. The ratio of width to length in the young stages, based

on an average of twenty measurements of young cotyledons, is 1.84 mm. to 2.55 mm. The ratio of width to length in the older stages is 4.63 mm. to 4.85 mm., based on twenty measurements of mature cotyledons. These ratios, expressed in percentages, are 0.722 and 0.954 respectively. The tips of the cotyledons are retuse to emarginate, with a peculiar narrowing of the blade just behind the tip.

Under favorable conditions, seeds of *Digitalis* germinate in from five to eight days, seeds of pure species germinating more quickly than seeds resulting from a species cross. The cotyledons reach maturity in from three to four weeks from the date of planting. The

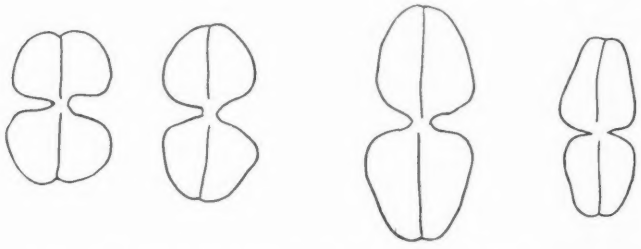

FIG. 2.—Young cotyledons about two weeks from date of planting, arrangement as in fig. 1; the *D. lutea*×*D. purpurea* F_1 hybrid has cotyledons decidedly larger than its reciprocal.

F_1 seedlings of *D. purpurea*×*D. lutea*, and those of the reciprocal combination *D. lutea*×*D. purpurea*, were carefully compared with each other, and with the parents in the very early stages, and these observations were continued at intervals of about one week from the date of planting to full maturity.

The cotyledons of *D. purpurea*×*D. lutea* are orbicular-elliptic in shape, greatly resembling those of *D. purpurea*, but showing the influence of the *D. lutea* parent to a slight extent. The ratio of width to length in the young stages of the cotyledons of these F_1 hybrids, based upon an average of twelve measurements, is 2.46 mm. to 2.58 mm. The ratio expressed in percentage is 0.961. The slight influence of the *D. lutea* parent is expressed in the slight elongation of the cotyledon. The seedlings of *D. purpurea*, which in this instance is the female parent, have cotyledons, which even in the

young stages are slightly broader than long, the ratio being 1.26 mm. to 1.21 mm., or 1.045 per cent. The resemblance in both shape and size of these F_1 hybrids to the female parent is maintained to full maturity, but decreases with the full development. At maturity in the F_1 hybrids the ratio of width to length, based upon the average of twenty measurements of mature cotyledons, is 4.413 to 4.3125. This ratio expressed in percentage is 1.023 (table I). This ratio, as well as the shape (figs. 3, 4), indicates that in this hybrid combination the

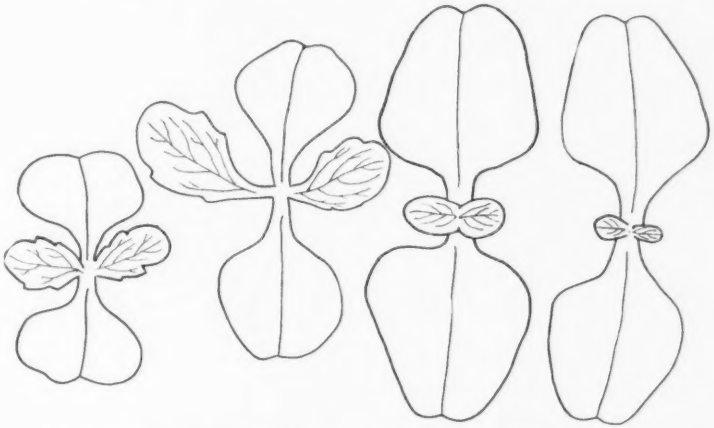

FIG. 3.—Mature cotyledons about three weeks from date of planting, arrangement as in fig. 1; true leaves beginning to appear; difference in size conspicuous in cotyledons at this stage.

matroclinous tendency as regards the form of the cotyledon is greater in the younger stages, for the cotyledons of the female parent are much broader than long. As the seedlings develop, the cotyledons remain smaller in this combination than in the reciprocal, and in general are about the size of the *D. purpurea* parent and much smaller than the *D. lutea* parent. As will be mentioned later, the feature of size is in sharp contrast with the reciprocal hybrid. At the end of two weeks the cotyledons of the F_1 hybrids of *D. purpurea* × *D. lutea* are scarcely more than one-half the size of the reciprocal. This difference in size occurred in several sets of sibs, the result of different hybridizations, and in one instance the F_1 hybrid seedlings were

even smaller than the *D. purpurea* seed parent. In general, the seedlings of this cross were about the same size as the seed parent in the earlier stages. They become slightly larger than the seed parent as they reach maturity.

Throughout their development from the earliest stages to full maturity, the cotyledons of the F_1 hybrid of *D. lutea*×*D. purpurea*, the reciprocal of the preceding combination, greatly resemble the *D. lutea*, which in this case is the female or seed parent. In both form

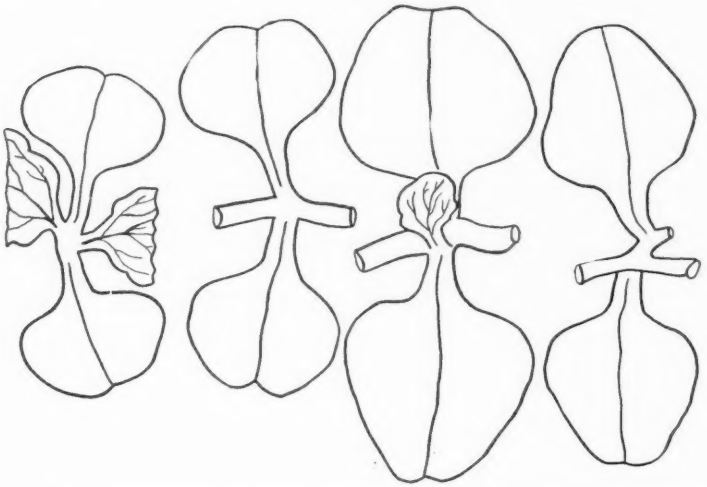

FIG. 4.—Fully mature cotyledons about four weeks from date of planting; arrangement as in fig. 1; petioles of true leaves shown; differences in size of cotyledons in reciprocals maintained to full maturity.

and size the resemblance of the cotyledons of these hybrids to the female parent is striking. In shape the cotyledons of the F_1 seedlings of this combination are elliptic in the younger stages, and become ovate to broadly ovate at maturity. In the younger stages, one week to nine days old, the cotyledons are elongated and appear to be almost identical with the *D. lutea* parent (figs. 1, 2). The ratio of width to length in young stages is 3.88 mm. to 3.84 mm., or 0.88 per cent, based upon an average of ten measurements. This compares with a ratio of 1.84 mm. to 2.55 mm., or 0.722 per cent for the *D. lutea*

parent. A slight influence in the F_1 hybrid of the *D. purpurea* parent, in which the cotyledons are broader than long, is expressed in a comparative shortening of the cotyledons. As full maturity is reached, the cotyledons become more nearly like the *D. lutea* parent (figs. 3, 4). The ratio of width to length, based upon the average of seven measurements of mature cotyledons, is 5.58 mm. to 5.67. Expressed in percentage, this ratio is 0.984. This compares with a ratio of 4.63 mm. to 4.85 mm., or 0.954 per cent for the *D. lutea* parent (table I). The figures and table I, showing the ratios of width to length, furnish convincing evidence of the striking resemblance of this hybrid combination to the maternal type as regards cotyledon form.

In size, the seedlings of F_1 hybrids of *D. lutea* × *D. purpurea* are larger and more vigorous than the reciprocal combination, and at maturity they are larger than the *D. lutea* parent. This disparity in size in the reciprocals is noticeable in all stages of development, and especially so after about two weeks from planting. It is interesting to note that the matroclinous tendencies so strong in the cotyledons are not extended to the true leaves of the seedling. The third, fourth, and fifth leaves were observed in the seedlings of reciprocal hybrids of *D. purpurea* and *D. lutea*, and in all cases the leaves of the seedlings in reciprocals were identical.

TABLE I

COTYLEDON WIDTH AND LENGTH IN PARENT SPECIES AND RECIPROCAL F_1 HYBRIDS

Stage	D. purpurea			D. purpurea × D. lutea F_1 hybrid			D. lutea × D. purpurea F_1 hybrid			D. lutea		
	Width in mm.	Length in mm.	Ratio width to length in percentage	Width in mm.	Length in mm.	Ratio width to length in percentage	Width in mm.	Length in mm.	Ratio width to length in percentage	Width in mm.	Length in mm.	Ratio width to length in percentage
Young	1.26	1.21	1.045	2.46	2.55	0.961	3.38	3.84	0.880	1.84	2.55	0.722
Mature	5.25	4.016	1.306	4.413	4.3125	1.023	5.58	5.67	0.984	4.6275	4.85	0.954

I have grown reciprocal hybrids of this combination to maturity and found the mature reciprocal hybrids to be identical. The *D. lutea* type seems to be largely dominant in shape of leaf, but the plants are intermediate in many respects. The matroclinous tendencies, therefore, stop abruptly with the maturity of the cotyledons. The peculiarity of the inheritance of cotyledon size and form is the only instance of matrocliny observed in *Digitalis* species hybrids, and I do not regard matroclinous tendencies as characteristic of F_1 hybrids of species of this genus.

It would be interesting to follow the inheritance of the cotyledon form and size through the F_2 and later generations, but due to the extreme sterility of *Digitalis* F_1 interspecific hybrids, the hybrids of this genus do not furnish suitable material for the study of cotyledons in later generations. In all the mass of plant genetical material now under investigation in the various laboratories, there should be some species, varieties, or strains showing differing cotyledon characters and yielding fertile F_1 hybrids. Such forms might furnish suitable material for the further study of the inheritance of cotyledon characters.[4] Doubtless data are already in the hands of investigators concerning cotyledon characters.

Discussion

Naturally the question of the cause of the matroclinous tendency of the cotyledon form and size in these F_1 hybrids arises. The situation is not parallel to those instances in animal forms, in which the hybrids of wide crosses show matroclinous tendencies. In the sea urchins of the echinoderms, where wide crosses are made, embryos of purely maternal characteristics result. The cause of this condition is the entire failure of the sperm to function in any capacity save as that of an activating agent. NEWMAN[5] states that this matroclinous situation is responsible for the traditional belief in the parthogenetic nature of heterogenetic hybrids, and has shown that it does not ob-

[4] Since the preparation of this manuscript, through the cooperation of Dr. SINNOTT and Mr. DURHAM, I have secured hybrids of homozygous strains of squash differing in cotyledon size, of which a study is being made.

[5] NEWMAN, H. H., Hybrids between *Fundulus* and *Mackerel*. Jour. Exp. Zool. **26**: 391–422 1918.

tain in that group of fishes known as the teleosts, in which the F_1s are true hybrids. The *Digitalis* hybrids under consideration are true hybrids, as shown by their growth to full maturity upon two different occasions.

In other instances, certain maternal characteristics in the early stages of the embryo of wide crosses in animal forms are accounted for on the basis of failure of the paternal chromatin to be assimilated in time to influence the developing embryo. This explanation has been made especially for the rate of growth of the embryo, or the "cleavage tempo."[6] NEWMAN's work, however, would seem to raise doubts as to the universal application of this principle. In any event, this explanation could scarcely be applied to the characteristics of mature cotyledons of plant embryos, for these are very late embryonic stages. Further, the third, fourth, fifth, and later leaves show perfect intermediate characteristics. Even the cotyledons, as is indicated, show to some extent the influence of the paternal parent in form.

It has been suggested to me by Dr. G. H. SHULL that the cause lies in the particular manner in which the seed coats, by compressing the cotyledons, determine their shape. The seed coats being formed from the integuments of the ovule, are entirely maternal in structure, and wholly uninfluenced by the hybridization which forms the embryo. The size of the seed and possibly the size of the embryo are apparently not unrelated to the size of the ovule. The size of the seed of *D. purpurea* differs from the size of that of *D. lutea*, the latter being three or four times the size of that of *D. purpurea*. The mature seed of *Digitalis* is well supplied with endosperm, however, which surrounds the embryo. In the developing stages the endosperm is soft tissue, and there may be objections to the idea of its compressing the embryo sufficiently to affect permanently the shape of the cotyledons of the embryo, even though the hard seed coats were restricting the whole developing structure.

Possibly the exact cause of the matroclinous tendencies of the cotyledons of these hybrids awaits further studies of the genetics of juvenile stages of plants, upon which there are as yet few data.

[6] MORGAN, T. H., Heredity of embryonic characters. Sci. Mo. 18: 5–17. 1924.

Summary

1. There are two distinct types of cotyledons in the seedlings of the various species of *Digitalis*. *D. purpurea* and *D. Thapsi* are characterized by cotyledons orbicular in shape, generally broader than long. *D. ambigua* and *D. lutea* and others are characterized by cotyledons elliptic to ovate, becoming broadly ovate at maturity, generally longer than broad.

2. F_1 hybrids between species with these different types of cotyledons show strong matroclinous tendencies. Matrocliny is not complete, since the influence of the pollen parent is seen in the ratio of width to length of the cotyledons.

3. The resemblance of the cotyledons of the F_1 hybrids to the seed or female parent is maintained in both form and size to full maturity.

4. Matrocliny does not extend to the true leaves in these hybrids. The third, fourth, and fifth leaves are intermediate and identical in reciprocals. Mature plants are identical in reciprocal hybrids of this combination.

5. This is the only instance of matrocliny in *Digitalis* species hybrids observed by the writer.

6. It is impossible to continue the study of cotyledon characters in these hybrids to the F_2 and subsequent generations, due to the extreme sterility of *Digitalis* species hybrids. Other plant forms might furnish suitable material for a more extended treatment of the topic.

7. The explanation of the causes of matrocliny in the cotyledons of F_1 hybrids between certain *Digitalis* species is difficult. The situation scarcely parallels cases in the lower animal forms. The most probable explanation is the relation of the purely maternal seed coats to the developing embryo. The subjection of the embryo to particular pressures in the different species may explain the form of the cotyledons. The size of the cotyledon may be in proportion to the size of the seed, which differs in the various species.

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THE "SPRUCE BUDWORM" BIOCOENOSE

I. FROST RINGS AS INDICATORS OF THE CHRONOLOGY OF SPECIFIC BIOLOGICAL EVENTS

I. W. Bailey

(WITH PLATES IX–XI)

Introduction

Analyses of the growth rings in stems of spruce and fir balsam have proved of considerable significance in studying the disastrous activities of the budworm, *Cacoecia fumiferana*, in the coniferous forests of Maine and eastern Canada. Such stem analyses, for example, have provided the most reliable method of dating the advent of serious infestations in various forest areas. In addition, they have afforded useful criteria in estimating the quantitative effects of varying intensities and durations of defoliation upon the growth and productivity of trees in sample plots.

As might naturally be expected, defoliation by the budworm leads to the formation of more or less abnormal growth layers in the secondary xylem. In the apical portions of the stem cambial activity usually is reduced, even during the first year of feeding, but some wood tends to be formed during each succeeding growing season until the tree dies or recovers. On the contrary, in the basal portions of the stem of seriously defoliated trees the cambium frequently forms an unusually wide ring during the first year, but subsequently may become inactive during one or more growing seasons. Under favorable circumstances, the patterns of abnormal rings are clearly differentiated. Thus, by comparing series of disks from the stems of many trees, and by rejecting all doubtful specimens, Craighead (**6**) has succeeded in tracing the chronology of successive outbreaks of the budworm in eastern Canada.

In conducting more detailed investigations in specific localities, numerous difficulties are encountered in dating the growth layers in certain trees. Owing to variations in the width of annual rings, due to climatic and other environmental factors, it frequently is difficult to determine whether a particular growth layer was formed

during, prior to, or subsequent to the first feeding of the budworm. For example, in figs. 1 and 6 the narrower rings are undoubtedly due to defoliation, but it is not evident which of the three subtending growth layers was formed during the first year of feeding. Each is slightly narrower than the ring which subtends it, indicating a reduction of cambial activity, which, however, may be due to some other factor than defoliation. It is not possible to assign specific dates to these rings by counting backward from the outermost (1922) ring, since one or more of the narrower growth layers may be "false" rings. Moreover, it is not possible to obviate the uncertainty by tracing the rings downward into the basal portions of the stem, for the disappearance of one or more growth layers in basal sections (fig. 2) may be due to arrested cambial activity rather than to the presence of false rings in the terminal sections. It is essential in such cases to obtain some means of accurately dating one of the inner growth layers.

At the request of the Entomological Branch of the Canadian Department of Agriculture, the writer has devoted some attention to the investigation of certain structural abnormalities which are significant in detecting the presence of false rings and in dating specific growth layers. The abnormalities were noted by CRAIGHEAD, and, since they appeared to be more or less closely correlated with the attacks of the budworm, were sent by him to the writer for detailed microscopic examination. They are of two distinct types, dark, reddish brown zones of varying widths, and yellowish brown rings of a somewhat "corky" appearance. The former (fig. 16) are zonal aggregations of "resin" cells and "resin" cysts, which are induced by the feeding of the budworm, as will be shown in the next paper of this series. The latter (figs. 1, 3, 9, 12, 13) are layers of compressed and distorted tissue and will be discussed in the following pages.

Structure of frost rings in fir balsam

As shown in fig. 12, the rings of distorted tissue consist in part of crumpled tracheids and in part of crushed, undifferentiated, centripetal derivatives of the cambium. In the zone of compression, the rays are laterally displaced or buckled, and are inflated by an enlargement of their constituent cells. Such abnormalities are not

produced by mere defoliation, but are due primarily to mechanical rather than to nutritional disturbances. That they are not induced by the feeding of the budworm is shown also by their occurrence in growth layers formed many years prior to the advent of the insect. They are typical effects of severe frosts upon young, actively growing shoots.

CASPARY (1) demonstrated that contractions induced by low temperatures vary considerably in different tissues. As suggested by SORAUER (5), the greater peripheral contraction of the external tissues during the duration of a frost compresses the delicate cells in the cambial regions, which tend to become deformed. Furthermore, excessive tangential shrinkage in the zone of compression leads to the production of radial cracks or clefts (fig. 13). When the frost terminates and the tissues expand, a normal equilibrium of forces is not restored. Owing to more or less permanent deformations of the tissues, the radial and tangential stresses in the cambial zone are subnormal. This results in an abnormal enlargement and division of the surviving cells, which crowd toward areas of lessened radial and tangential resistance, and thus occlude many of the clefts in the zone of compression. The rays expand tangentially (figs. 15, 17), and the intervening cells frequently tend to differentiate into an irregular short celled parenchyma (figs. 13, 15). As growth continues the stresses are equalized, and the cells assume a normal form and radial seriation.

Although the abnormalities produced by low temperatures are fundamentally similar, their structural details vary in different species of plants (figs. 6–17), and in different representatives of a single species, depending upon the severity and duration of the frosts and the dates of their occurrence (figs. 12, 13, 16). The most constant and characteristic histological features of the frost injuries in fir balsam are the buckling and lateral enlargement of the rays, and the more or less pronounced distortion of the intervening cells; conspicuous radial clefts are not invariably present. The transitions between the zones of compressed tissue and the subsequently formed normal secondary xylem usually are abrupt (figs. 12, 16). Broad intermediate zones of traumatic parenchyma, emphasized by HARTIG (2), SORAUER (5), MIX (3), and others in their investigations of frost injuries, are of relatively infrequent occurrence. In young,

vigorous, actively growing shoots, having wide zones of cambial derivatives in various stages of differentiation, severe frosts tend to produce clearly defined, concentric layers of compressed tissue (fig. 12). There is an inner, broad zone of crumpled, unlignified tracheids, and an outer, narrower zone of crushed and more or less discolored, undifferentiated cells. In the case of milder frosts and of less rapidly growing shoots, these compressed cells may be reduced in number (fig. 16), or may be sporadically distributed, and thus fail to exhibit a striking zonal continuity.

Distribution of frost rings in fir balsam

The frost rings in stems of fir balsam examined by the writer are confined to the 6–8 innermost growth layers, that is, those in relatively close proximity to the pith. In other words, the stem at any particular level remains susceptible to injury during the first 6–8 seasons of cambial activity. Owing to the fact that each zone of cambial derivatives jackets the previously formed ones and extends beyond them in the apical portion of the stem, the successive frost injuries occur at higher and higher levels. Their longitudinal extension varies in different trees and in different portions of a single cauline axis. This is due in part to fluctuations in the rate of elongation of the terminal shoot. The intervals between the growing points and the levels at which the secondary tissues lose their susceptibility to frost injuries are lengthened in rapidly elongating shoots and are shortened in slowly growing ones.

It is significant in this connection, however, that cambial activity does not always begin simultaneously, or progress at a uniform rate in all trees, or even in all portions of one stem. The condition of the tissues at the dates upon which low temperatures occur must have a more or less marked effect, therefore, not only upon the detailed structure of the resulting frost rings, but also upon their distribution in specific trees or growth layers. For example, the effects of frosts occurring at the beginning of the growing season may be curtailed by cambial inactivity in portions of stems which are not immune to later frosts. The most extensive and clearly defined injuries are those produced by unusually severe late frosts during periods of general, accelerated secondary growth.

As noted by RHOADS (4) in his investigation of various western

Coniferae, the position of frost rings in transverse sections of stems varies considerably in different growth layers. They commonly are located in the first formed portion of these layers, but may occur at times in their median portions, or rarely even upon their outer margins. Such variations in the location of the injuries are obviously determined by fluctuations in the date of the occurrence of low temperatures during different growing seasons. In certain cases, two or even three frost rings (fig. 13) may be present in a single growth layer.

Significance of frost rings in dating specific growth layers

In view of the fact that frost injuries may readily be distinguished under a hand lens by anyone familiar with their peculiar color and irregular contour, it occurred to the writer that they might prove to be of considerable significance in cross correlating homologous layers in different trees, and thus in accurately dating the growth rings in doubtful cases. An examination of fir balsams from CRAIGHEAD's "Fief St. Claire" and "Saguenay" sample plots in south central Quebec indicated that such is at times the case, and justified a series of more detailed investigations at Long Lake, western Quebec, and near Bathurst, northern New Brunswick.

In each of these regions, the injuries induced by certain specific frosts (unusually severe late frosts occurring during periods of general acceleration of cambial activity) are widely distributed in the vegetation. By sectioning the stems of fir balsams at appropriate levels, it is possible to locate these injuries and to use them as bases in verifying the chronology of growth layers formed during and subsequent to the feeding of the budworm. Most of the fir balsams in the St. Claire and Saguenay sample plots are characterized by having a more or less conspicuous frost injury in the upper extension of their 1910 ring, and those in the Long Lake and Bathurst sample plots by having similar injuries respectively in their 1918 and 1914 growth layers. In south central Quebec the first feeding of the budworm occurred in 1911, at Bathurst in 1914, and at Long Lake in 1918.

As previously stated, it frequently is difficult to differentiate the growth layers formed during the first year of feeding in such stems as those illustrated in figs. 1–6, since false rings may be present in the apical sections and one or more rings may be missing in the

basal sections. The position of the frost injuries in the apical sections, however, may be utilized in obviating such uncertainties as these. Fig. 1 shows the sequence of rings in the terminal portion of a balsam from Fief St. Claire. That the next to the innermost layer is the 1910 ring is indicated by the zone of crushed and distorted tissue near its inner margin. Thus the succeeding layer must have been formed during the first year of feeding of the budworm, that is, 1911. Furthermore, there are eleven intervening layers between the 1910 ring and the outermost or 1922 ring; one for each of the 1911–1921 growing seasons. It is evident, accordingly, that none of the narrower growth layers are false rings. Having dated the rings in the apical portion of the stem, it is possible to trace them downward into its basal portion. The innermost layer in fig. 2 is the basal extension of the 1910 ring. There are only nine succeeding layers, the outermost of which is the 1922 ring. In other words, the cambium remained inactive at the base of the stem during three growing seasons of the 1913–1918 period. In fig. 6, an apical section of a balsam from Bathurst, the position of the 1914 ring is determined by a narrow zone of crumpled and inflated ray tissue. This growth layer, which was formed during the first year of feeding of the budworm, is separated from the outermost (1922) ring by eight intervening rings of varying widths. One of these layers must be a false ring, since there are but seven growing seasons between 1914 and 1922. Under higher magnification, it may be demonstrated that the 1917 layer is divided into two rings by a narrow zone of traumatic "resin" cells, which simulates the outer boundary of a true growth layer. Figs. 4 and 5 are sections of the stem of a balsam from Long Lake. In the section from the upper portion of the stem, there are two wide inner layers and what appear under a hand lens to be four narrow outer layers. The narrower of the two inner layers was originally considered to have been formed during 1918, the first year of feeding of the budworm at Long Lake. In the basal section, the two narrow outer layers are subtended by an unusually wide ring. The enlargement of this ring was interpreted as due to accelerated cambial activity during the first year of defoliation, 1918. Subsequently an additional section was cut at a higher level than that shown in fig. 4. In this section (fig. 3) there are five narrow outer rings. If none of these layers is a false ring, the innermost ring in

fig. 4, which is a basal extension of the corresponding ring in fig. 3, must be the 1918 growth layer. That such is indeed the case is indicated by the position of the frost injury in fig. 3. The 1921 ring in fig. 4 is so tenuous as to be indistinguishable under a hand lens. Furthermore, the wide ring in fig. 5 is not a basal extension of the outermost of the two wide layers in fig. 4, but of the succeeding (1918) ring.

It should be emphasized in conclusion that, in utilizing frost injuries as a means of dating the growth rings in the trees of a specific region, it is essential to determine whether the effects of particular frosts are sufficiently characteristic and widely distributed in the vegetation to justify their use as indicators in stem analyses of individual trees. This involves the examination of very considerable material. In the case of relatively recent outbreaks of the budworm, such as those with which we are at present concerned, the significant injuries fortunately are those which occur in young plants and in the slender terminal portions of old stems. The task of tracing their distribution is not particularly laborious or difficult, therefore, since it does not involve the dissection and detailed examination of the more robust portions of old stems. Furthermore, it is of interest in this connection that the effects of late frosts are visible in the stems of dicotyledons (figs. 7, 8, 10, 11, 14, 15, 17). It is possible to make a preliminary survey of the distribution of frost injuries in such commercially worthless plants as *Alnus*, *Corylus*, *Salix*, *Prunus*, *Sorbus*, etc., and thus reduce the number of potentially valuable young balsams that must be felled and examined.

A reconnaissance of this character proved to be particularly valuable at Long Lake, western Quebec, a region where late frosts are of almost annual occurrence. An examination of a large number of stems of various dicotyledons revealed a characteristic pattern of frost injuries. In other words, the frost rings formed during the 1913–1915, 1918, and 1922 growing seasons proved to be very clearly differentiated and widely distributed in the dicotyledonous vegetation. That more or less severe frosts occurred during the intervening growing seasons was indicated by the presence of additional frost injuries in the stems of the more susceptible species, for example *Sorbus* (fig. 7), but the effects of these frosts were found to be of extremely sporadic occurrence in the less susceptible dicotyledons.

Even in the case of the mountain ash, the 1913–1915, 1918, and 1922 frost rings could readily be distinguished from the intervening frost injuries by characteristic differences in their position, form, and severity (fig. 7). It was subsequently determined that similar patterns of frost injuries occurred in stems of fir balsams from CRAIGHEAD's sample plots, and, therefore, it was possible to utilize the conspicuous 1913–1915 and 1918 frost rings as indicators in verifying the chronology of the outermost growth layers.

Summary and conclusions

1. In studying the more or less abnormal growth layers in the stems of conifers defoliated by the spruce budworm, it is difficult in many cases to determine which rings were formed during specific growing seasons. This is due to the frequent omission of one or more growth layers, particularly in the basal portions of the stem, and to the occurrence of structures resembling false rings.

2. A reconnaissance of frost rings in the vegetation of various forest areas in eastern Canada indicates that the injuries produced by certain frosts are widely distributed in the stems of fir balsam, alder, hazel, birch, willow, poplar, cherry, mountain ash, etc. They afford a reliable means of cross correlating homologous growth layers in different trees, and, therefore, of accurately dating specific growth layers in fir balsams defoliated by the budworm.

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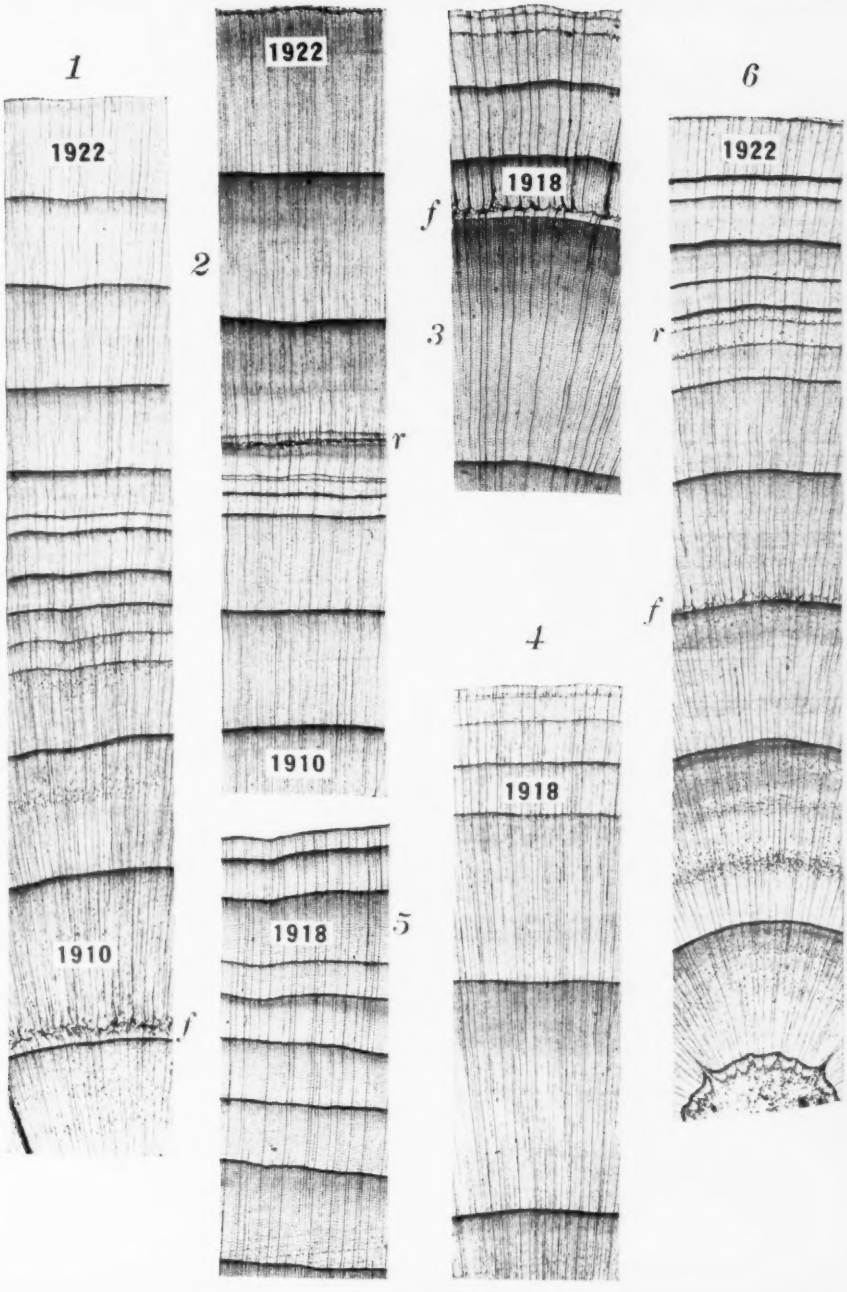
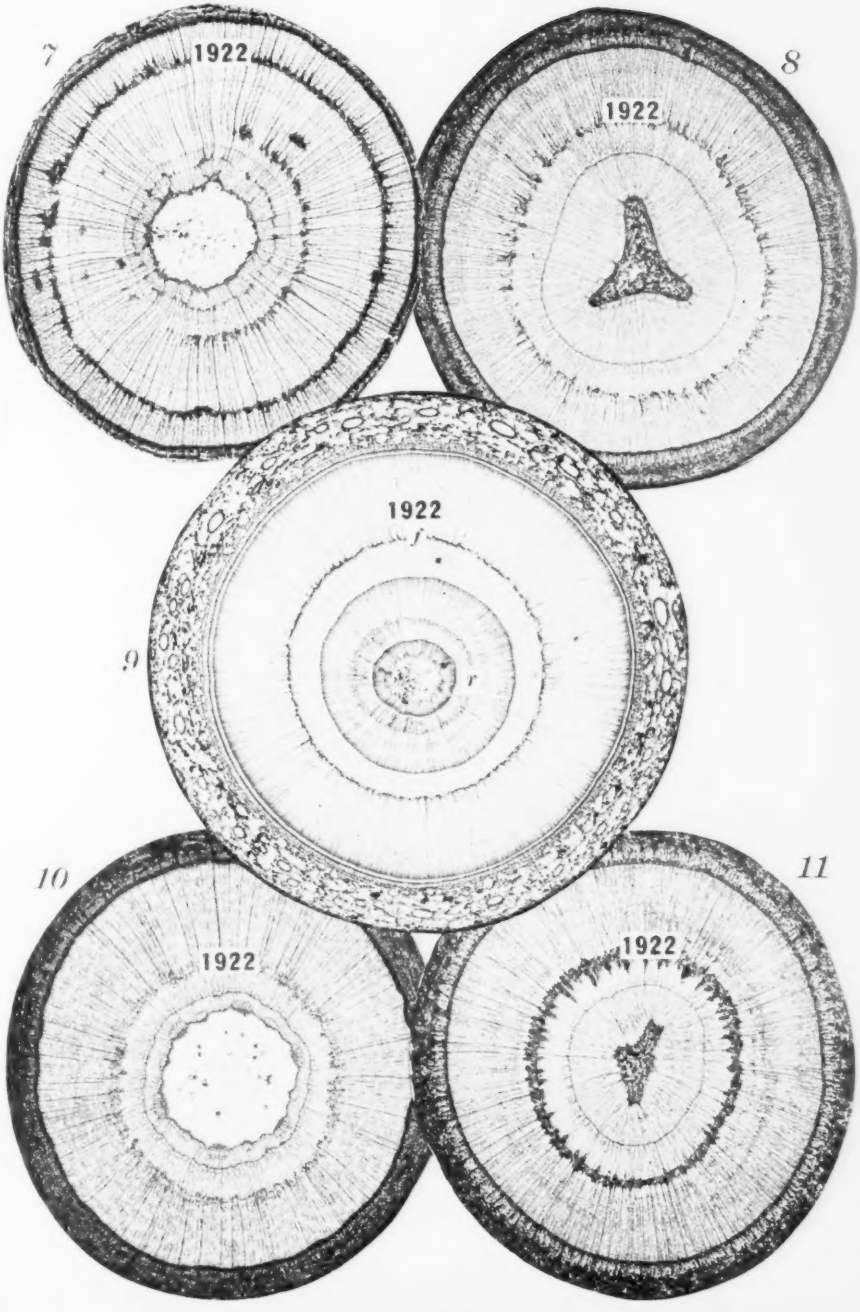
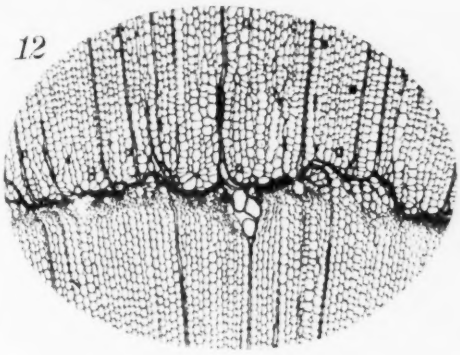
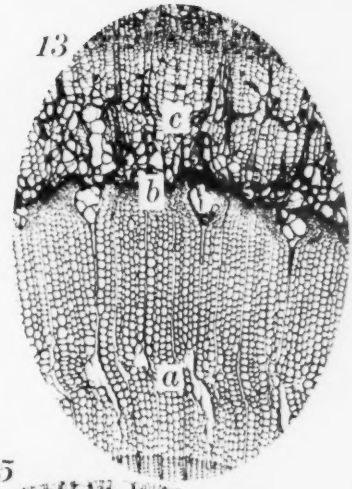


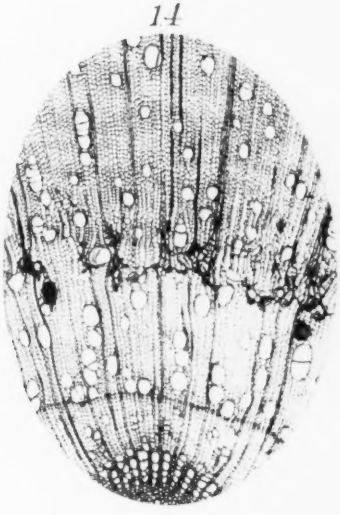
BAILEY on FROST RINGS

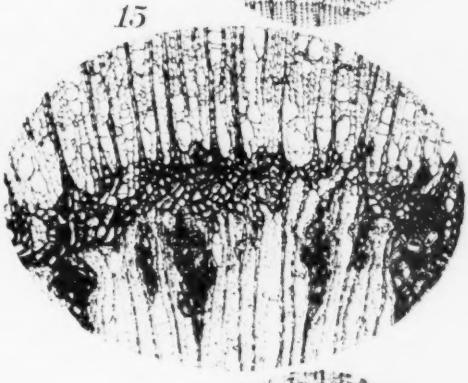


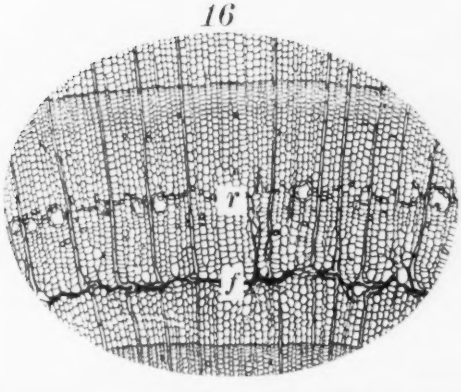
BAILEY on FROST RINGS

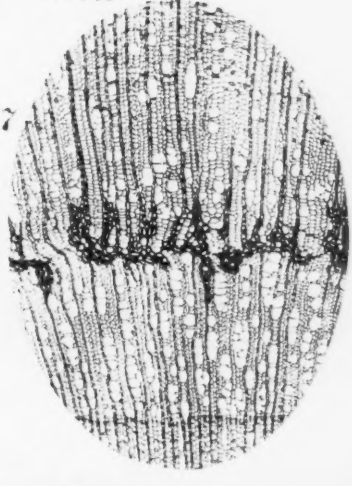












BAILEY on FROST RINGS

DESCRIPTION OF PLATES IX–XI

FIG. 1.—*Abies balsamea:* transverse section of terminal portion of old stem, showing pattern of abnormal growth layers in tree which recovered from attacks of budworm; *f*, frost injury; first feeding of budworm in 1911; ×7.

FIG. 2.—Same: transverse section of basal portion of same stem, showing omission of three growth rings during 1913–1918 growing seasons; *r*, arc of traumatic "resin" cysts induced by feeding of *Pissodes dubius;* ×9.

FIG. 3.—Same: transverse section of terminal portion of old stem, showing pattern of abnormal growth layers in dying tree; *f*, frost injury; first feeding of budworm in 1918; ×15.

FIG. 4.—Same: transverse section of same stem, cut at slightly lower level than that shown in fig. 3; ×10.

FIG. 5.—Same: transverse section of basal portion of same tree, showing omission of 1921 and 1922 growth layers and marked acceleration of cambial activity during 1918; ×10.

FIG. 6.—Same: transverse section of terminal portion of old stem, showing pattern of abnormal growth layers; *f*, frost ring; *r*, ring of traumatic "resin" cells, simulating outer boundary of growth layer; first feeding of budworm in 1914; ×7.

FIG. 7.—*Sorbus americana* (Marsh.) DC.: transverse section of terminal shoot of young plant, showing effects of 1920, 1921, and 1922 frosts: ×12.

FIG. 8.—*Alnus incana* (L.) Moench.: transverse section of stem of young plant, showing characteristic 1922 frost injury; ×11.

FIG. 9.—*Abies balsamea:* transverse section of terminal shoot of young plant, showing characteristic 1922 frost injury; *f*, frost injury; *r*, ring of traumatic "resin" tissue; ×7.

FIG. 10.—*Salix* sp.: transverse section of young stem, showing characteristic 1922 frost injury; ×11.

FIG. 11.—*Betula alba* L. var. *papyrifera* (Marsh.) Spach.: transverse section of terminal shoot of young plant, showing characteristic 1922 frost injury; ×8.

FIG. 12.—*Abies balsamea:* portion of fig. 9 more highly magnified, showing zones of compressed tissue and buckling of rays; ×46.

FIG. 13.—Same: transverse section of terminal shoot of young plant, showing the effects of three successive frosts; at *a* radial clefts and slight distortion of tracheids; at *b* zones of compressed tissue, "inflated rays," and zone of traumatic parenchyma; at *c* narrow, irregular zone of compression; ×50.

FIG. 14.—*Salix* sp.: portion of fig. 10 more highly magnified, showing zone of compression and distorted tissue; ×45.

FIG. 15.—*Betula alba* var. *papyrifera:* portion of fig. 11 more highly magnified, showing zone of compression, "inflated rays," and outer zone of traumatic parenchyma; ×45.

FIG. 16.—*Abies balsamea*: transverse section of wood, showing frost ring at *f*, and zone of traumatic "resin" tissue at *r;* ×45.

FIG. 17.—*Alnus incana:* portion of fig. 8 more highly magnified, showing buckling and enlargement of rays; ×50.

HYDROLYTIC ENZYMES IN PHORMIDIUM LAMINOSUM

OLGA LAKELA

Little work has been done on the occurrence of enzymes in the blue-green algae, in comparison with the extensive research work done on the enzymes of bacteria, fungi, and higher plants. According to GREEN (2), lipases occur in oil bearing seeds. CAMUS (1) reports them in higher and lower fungi, and they also have been found in various bacteria.

Amylases are widely distributed in the plant kingdom. As early as 1814 KIRCHOW observed the decomposition of starch in germinating barley. The isolation of the enzyme in concentrated form was accomplished in 1853 by PAYEN and PERSOZ, who named it diastase. Since then many amyloclastic enzymes have been detected in many plant products. Invertase occurs in many fruits, in germinating pollen grains, in some fungi, and in bacteria. VINES (9) discovered proteolytic enzymes in seeds, leaves, in certain juicy fruits, and in insectivorous plants. They have also been observed in fungi and bacteria.

It has commonly been believed that certain of the enzymes occur universally (3), and that the metabolic processes of a living organism are controlled by the presence or the activity of these enzymes. The enzymes present in an organism may determine the substrate in which it can grow. The presence of these common biological catalysts, however, is not necessary for the vital reactions if the syntheses or hydrolyses which they catalyse under ordinary conditions can be brought about by physical or chemical conditions such as high temperature or acidity.

This study was undertaken to demonstrate the importance of enzymatic action in the life processes of an alga which lives above the maximal temperature for most enzymes. The investigation showed the absence of some of the most common enzymes in plants. A chemical analysis of the algal material was also undertaken, to determine what substances were present in the metabolism of which enzymatic activity would be of importance. It is difficult to perceive

in the scale of evolution how organisms could start with a full complement of enzymes, since in many cases these have been shown to be complex and labile substances. It would seem more logical to assume that the extremely primitive organisms might equally well be dependent upon the rates of reactions, which are determined by external conditions such as the temperature, rather than dependent upon the complex and delicate catalysts such as many enzymes are known to be. The thermal algae are known to be very primitive with regard to morphological organization and in their physiological processes. Many of them live under conditions for nutrition such as may have prevailed at the time of the origin of living things. Their adaptation to temperature as high as 89° C. indicates that their ecological requirements are much different from those of many higher plants in this regard.

The collections of algae used in this study were made from Hymen Terrace Spring at Yellowstone National Park by Dr. R. B. HARVEY in June, 1923. The temperature of the spring water at the time of collection registered 73°–74° C. The material was preserved either with toluol or with 80 per cent alcohol. The alga was identified as *Phormidium laminosum* (Ag.) as described by TILDEN (**5, 6**).

Diastase and invertase

For a determination of diastase and invertase in this algal material, one per cent solutions of sugar and of starch were prepared. The specimen preserved with toluol was used in tests of enzymatic activity. Since autolysis may have occurred in the material, the enzymatic activity of the preserving fluid, as well as the material itself, was determined. The mass of algal filaments with its mineral contents was well ground in a mortar, and a suspension in distilled water was prepared. To estimate the dry weight of the material taken for each experiment, an aliquot of the suspension was filtered, dried, and weighed. The tests were set up as follows:

SERIES I

1. 25 cc. starch 1 per cent+10 cc. filtered extract
2. 25 cc. starch 1 per cent+10 cc. suspension
3. 25 cc. sucrose 1 per cent+10 cc. filtered extract
4. 25 cc. sucrose 1 per cent+10 cc. suspension
5. 25 cc. distilled H_2O+10 cc. filtered extract
6. 25 cc. distilled H_2O+10 cc. suspension

The first four samples were left in an incubator for 30 minutes at 48° C. The values of 5 and 6 were determined directly. In determining the values for these solutions, the method described by THATCHER (**7, 8**) was used. For measuring the quantity of reducing sugars, PETER'S (**4**) iodide method was found to give the most satisfactory results.

The values of Fehling's solution, the starch, and sugar substrate used as controls checked with one another. In the incubated solutions some hydrolysis occurred. Sample 2, which contained the starch substrate, and the suspension produced 26.40 mg. of dextrose; however, this result is small enough to lie within the range of experimental error.

Further trials were carried out at a temperature of 74° C. The solutions were identical in composition with those of the first series, and the same methods were applied. Most reduction occurred again in sample 2, as in the identical one of the first series. This time the dextrose value was 9.20 mg., which was less than the value obtained at a lower temperature. The same was true of the other samples. If, therefore, the reduction in these experiments is due to the enzymatic activity, the activity decreases at the temperature of 74° C. The plant grows at a temperature of 74° C., however, and its enzymes, if present, might be expected to act at this temperature.

In the third series of trials, identical samples were subjected to a temperature of 74° C., for twenty-four hours. The titration values for the controls and the digested samples were identical Obviously in this series of trials the conditions for enzymatic activity were most favorable, but the results do not indicate any, so that the absence of diastase and invertase from *Phormidium* seems definitely established.

Proteose activity

Tests for tryptophane were determined according to the directions in A.O.A.C. Methods of Analysis (**11**). Experiments with casein substrate showed that *Phormidium* does not contain a casein splitting enzyme. Further trials were made to discover whether or not the algal material contains an enzyme capable of hydrolyzing its own protein. The plant material preserved in toluol was allowed to act on protein prepared from the material which had been preserved in alcohol. One set of samples was incubated at 48° C. for twenty-

four hours, another set at 74° C. for the same length of time. The test for tryptophane in all the samples was negative. Evidently some reaction such as that of VAN SLYKE will need to be used to determine proteose activity, since tryptophane seems deficient in the protein; but for such an experiment a sufficient quantity of the material was not available.

Lipase activity was determined according to the directions by SHARP and MORROW (12). In the digested samples a slight increase in acidity occurred, due to the presence of free fatty acids formed by lipase activity. To test the heat resistance of lipase, a series of experiments was carried out at temperatures from 74° to 95° C. Some interfering reaction, however, induced by high temperature, prevented the formation of free fatty acids, causing decreasing acidity. Nothing could be concluded as to the heat resistance of this enzyme.

Glycogen is a common carbohydrate in the blue-green algae. Since *Phormidium* belongs to this group, it was subjected to a test for a glycolytic enzyme. A substrate of 0.4 per cent glycogen was prepared, and the experiments were carried out according to the methods given under diastase and invertase activity. The dextrose value obtained was 9.55 mg. If this small amount of reduction is not due to experimental error, the action may be said to be due to a glycolytic enzyme.

Nutritive substance

A portion of the collection preserved in alcohol was used in tests for nutritive substances. The official methods (11) were applied. Reducing sugars or sucrose were not present. Organic nitrogen was determined by Kjeldahl's method. The protein represented 2.6 per cent on the basis of ash free substance. Free amino acids were found present in the material.

On acid hydrolysis the alcohol insoluble residue yielded 1.35 per cent of starch. It is questionable whether this value represents starch. Other polysaccharides probably in the wall may yield dextrose on acid hydrolysis. A microchemical study failed to show starch grains in the algal material. Glycogen was found to represent 0.5 per cent of the material on a dry weight basis. Pentosans were not present.

Summary

1. The alga growing in Hymen Terrace Spring at 74° C. was identified as *Phormidium laminosum* (Ag.).

2. The plant material contains 53 per cent of ash on dry weight basis.

3. The absence of diastase and invertase from *Phormidium* was definitely established

4. *Phormidium* does not contain a casein splitting enzyme; the plant protein does not contain tryptophane in detectable quantity.

5. Lipase activity was shown at 48° C.; at 74°–75° C. its activity could not be determined, on account of some interfering reaction which prevented the determination of free fatty acid production

6. Glycogen splitting enzyme was probably present.

7. *Phormidium* does not contain sugars, starch, or pentosans in detectable quantity.

8. Proteins, amino nitrogen, and glycogen were present

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ENLARGED BASES IN FRAXINUS NIGRA IN MICHIGAN[1]

FRANK C. GATES AND C. O. ERLANSON

(WITH TWO FIGURES)

In the northern part of the lower peninsula of Michigan, the lowlands in general are bogs or swamps in which *Thuja occidentalis* L. largely predominate in the natural vegetation. Following lumbering or burning, the new forest, especially along streams, frequently contained a high percentage of *Fraxinus nigra* Marsh. In extreme cases the groves might contain little but black ash. This is a perfectly normal habitat for this tree, as it grows readily in wet places from streams to undrained bogs. The striking thing about these trees growing along certain streams or in their immediate vicinity, however, is the base, which is enlarged very much more than seems to be necessary from any standpoint under consideration. These trees grow somewhat close together, so that they protect one another from the wind. Furthermore, the trees are deciduous and therefore less exposed to the severity of winter. None of the other trees present in such habitats have the striking enlargements of their bases.

The best examples of this enlargement occur in the delta of Maple River, just southwest of Colonial Point (fig. 1). The trees are well developed, approximately of the same age, and about 6–8 inches in diameter above the base. The diameter of the base itself is about 13–15 inches. The seedling trees that are now appearing in this area are usually red maple or white cedar rather than black ash. A second area in which the enlarged bases may be found is that at the southwestern corner of Smith's bog, in which, however, there are only a few such trees.

The root system of these trees is quite shallow, as is normally

[1] Contribution no. 232 from the Botanical Laboratory of the Kansas State Agricultural College and a contribution from the Biological Station of the University of Michigan. Mr. ERLANSON is responsible for the preparation and study of the wood, while the senior author is responsible for all other parts.

found in the case of trees growing in water. The changes in lake level are not sufficient to cover more than about one-third of the height of the enlarged bases. Frequently, after the trees have grown to the point of casting considerable shade, the ground plants beneath die out, and the surface soil breaks up and washes away, so that, in places in the immediate vicinity of the streams, one will find these trees apparently perched on top of the bottom, rather than beneath the soil under the water.

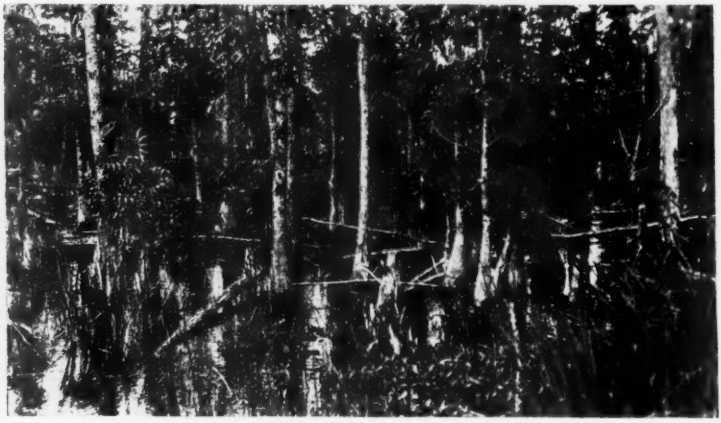

FIG. 1.—View of deltal region of Maple River, showing enlarged bases of *Fraxinus nigra*, August 8, 1921.

The general appearance of the above-ground parts of these ash trees is much the same as that of black ash trees growing in bogs. They differ only in that the branches are inclined to be rather short, although spreading widely from the trunk. The upper parts of the trees are frequently dead. At a distance the woods appear to be full of dead trees, with a growth of younger trees coming on but not quite topping the dead stems. This appearance, however, soon gives way to the true state of affairs as one enters the woods. The green shoots for the most part come from a little below the tops of the trees, and not from along the trunk in the vicinity of the base, or from younger trees. From the swollen base to the crown there is very little change in the thickness of the trunk, but at the top it narrows to an abrupt

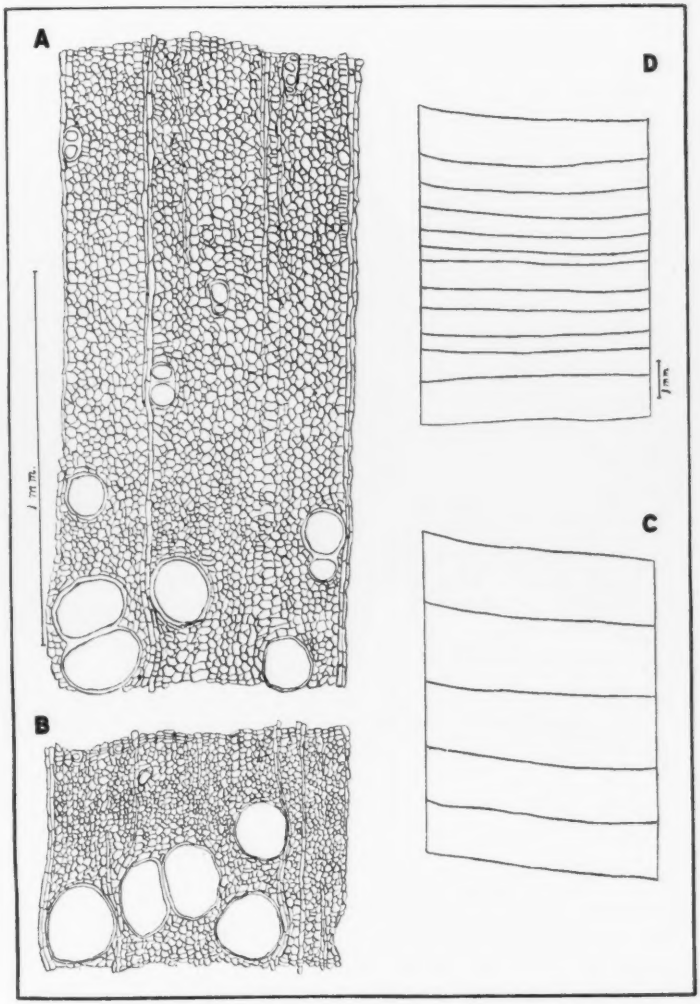

FIG. 2.—Sections of black ash trunk drawn with aid of projection apparatus: *A*, *B*, transverse sections of typical year's growth, *A*, taken from swollen base, *B* from normal trunk; *C*, *D*, transverse sections showing annual rings, *C* in swollen base, *D* in normal trunk (scale for *A* and *B* on left, and for *C* and *D* on right).

irregular conical shape. The swollen bases of the ash trees make the area very suggestive of swamps in the southern states, and not what one would expect to find in the north. Nothing resembling cypress knees, however, was discovered in the black ash groves.

Sections were made in three planes, 15 μ thick, from various points along the radius of the enlarged base. Sections were also made from the normal bole farther up on the tree, at corresponding positions to those taken below, so that comparisons could be made (fig. 2). Sections made at the center of the trunk show that for about the first thirty years the tree was normal throughout its trunk. The annual rings of the swollen part then gradually become farther apart up to a certain width, which is maintained, with small fluctuations, to the cambium. A distance of 3 cm. on the radius of the swollen part covers thirteen annual rings, whereas the same distance on the radius of the corresponding normal part covers thirty-three annual rings.

The average diameters of the elements in the swollen part do not differ from those in the normal part, nor are they in any way abnormal in shape. The number of large spring vessels, in transverse section of any fixed area of the swollen part, is the same as the corresponding area in the normal part. There is no increase in the number of vessels in the swollen part. The increase in the width of the swollen base is due to the great increase in the number of cells of the summer wood.

Summary

Specimens of *Fraxinus nigra* Marsh growing in permanently water covered areas adjacent to certain streams in Cheboygan County, Michigan, have a very conspicuously enlarged base, in most cases nearly twice that of the diameter of the trunk above.

The swollen base is due, mechanically, to the great increase in the number of cells of the summer wood of each year's growth, there being about twice as much growth in the swollen part as in the normal part. The individual cells of the swollen part are not abnormal either in size or shape.

KANSAS STATE AGRICULTURAL COLLEGE
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BRIEFER ARTICLES

MARK ALFRED CARLETON

(WITH PORTRAIT)

MARK ALFRED CARLETON died April 26, 1925, in Paita, Peru, from an attack of malaria. Although widely known by his work in vegetable pathology, CARLETON established his greatest record by his work on the introduction of new varieties of cereals into this country, and his death is a severe loss to American agriculture. He was born near Jerusalem in Monroe County, Ohio, in 1866. When he was ten years of age, his parents moved to a farm in Cloud County, Kansas. His early education was obtained in the rural schools of Ohio and Kansas. In 1884 he entered the Kansas Agricultural College at Manhattan, where he completed his course and also a year of special work in biology and chemistry, graduating with the degree of Bachelor of Science in 1887. During 1890–1891 he was Professor of Natural History in Garfield University at Wichita, Kansas; during 1891 and 1892 he taught natural history in Wichita University; and during 1892–1893 he took a postgraduate course in botany and horticulture at the Kansas Agricultural College, receiving the degree of Master of Science. During 1893 he was Assistant Botanist at the Kansas Experiment Station, giving special attention to plant pathology and particularly to cereal rusts.

In 1894 CARLETON began his service in the United States Department

of Agriculture, by appointment as Assistant Pathologist, his time being devoted chiefly to the study of cereal diseases. In 1901 he was appointed Cerealist in Charge of Grain Investigations in the Bureau of Plant Industry, a position which he held until May 8, 1918. During 1898 and 1899 he was an Agricultural Explorer in eastern Europe and Siberia, in search of rust-resistant and drought-resistant cereals. In 1900 he was Expert in Charge of the Grain Exhibit of the United States at the Paris Exposition, and in the same year he made another trip to eastern Europe in search of hardy cereals, and to increase the supply of those originally obtained.

Before entering the Department of Agriculture, CARLETON had published some reports on rusts of grains, some lists of Kansas parasitic fungi, and some observations on various flowering plants of Oklahoma, etc. It was under the auspices of the Department of Agriculture, however, that he published his great work in plant pathology, establishing the physiological relationships of nearly all the cereal rusts of this country, demonstrating the distinctness of the different forms of the same species adapted to the same host, and showing that durum wheats, emmer, einkorns, and some other wheats are more or less rust-resistant.

Having visited Russia and Siberia, CARLETON had acquired a thorough knowledge of the various cereals cultivated in these countries. Being a very keen observer, he selected a number of varieties suitable for cultivation in this country, and their introduction proved a great success. According to the Proceedings of the American Society of Agronomy (1910), the introduction of durum wheat means an annual yield of 60,000,000 bushels; the introduction of Swedish Select oat furnishes 40,000,000 bushels of the annual oat crop, etc. The introduction and establishment of these cereals was a very difficult task, and CARLETON had to make many experiments at the experiment farms in various parts of the country to test their hardiness and disease resistance. His very comprehensive paper, *The basis for the improvement of American wheats*, gives an idea of the magnitude of the work which he accomplished in this direction. It is really remarkable what an immense field CARLETON covered in so short a period as twenty-five years, especially considering his very modest education in natural science. Three years of Latin and one year of Greek constituted his fundamental knowledge of languages, and yet he undertook the difficult task of studying the Russian language before going abroad in search of new varieties of cereals.

After leaving the service of the United States Department of Agriculture in 1918, CARLETON undertook some important work with the Grain

Corporation of America. This was about the close of the World War, and the Grain Corporation of America had some very important economic problems in connection with cereals to handle. In this work he rendered excellent service. Leaving the Grain Corporation, he entered the service of the United Fruit Company, and was sent to Central America to make a study of banana diseases. He had headquarters for some time near Port Limon, and there developed a laboratory and conducted some important field investigations in the selection of disease-resistant bananas. In 1922 he severed his relations with the United Fruit Company and was called to British Honduras for pathological work in connection with another large fruit company of that country. He remained in British Honduras until December, 1924, when he went to Peru in connection with a large project having for its object the production of cotton and other crops in Peru, and was making special studies of cotton diseases at the time of his death.

CARLETON was a self-made man, full of energy and inspiration, and always active, and his keen eye for nature guided him to accomplish what he did. In the history of American botany his name will be recorded as one of the leading pathologists, whose work culminated in a result of such magnitude as to mark an epoch of the greatest importance to American agriculture.—THEO. HOLM, *Clinton, Md.*

NOTICE

Although the residence of the editor is changed from Chicago to Yonkers, N.Y., for the coming year, he will still have charge of this journal.

All communications and manuscripts should be addressed to **Editorial Office, Room 11A, Botany Building, University of Chicago.**—J. M. C.

CURRENT LITERATURE

BOOK REVIEWS

The cell

It is thirty years since the first edition of WILSON's work on the cell appeared. Four years later a second edition followed, which included considerable revision, especially in the section dealing with the centrosome, and which brought the literature up-to-date. No other book ever did so much to stimulate investigations upon the structure, development, and functions of the cell. This authoritative presentation enabled the younger generation of zoologists to undertake researches which would have been impossible without such aid. Botanists, having no similar presentation in their field, used WILSON's book, and thus obtained some knowledge of the animal cell and its problems. If such a book as SHARP's *Introduction to cytology* had appeared in the nineties, the zoologists of today might have had a better appreciation of the plant cell and its problems.

A new volume by WILSON[1] has now appeared, which is not really a revision of the earlier editions, but a new book, with the number of pages increased from 483 to 1232 and the number of illustrations from 194 to 529. The paper is thin and of such excellent quality that the illustrations are well reproduced, and the book is not unwieldy to handle.

In general, the book is written frankly from the standpoint of the zoological student of cytology and embryology, without pretence of competence to deal with outside fields; but the treatment of plant cells and botanical problems is much more accurate and extensive than in previous editions. While the botanist will still depend upon SHARP, the opinion of a competent zoologist in regard to structures which seem to be identical in plants and animals will always be helpful and suggestive.

WILSON's book is very different from the *General cytology*, recently published by the University of Chicago Press, which was written by thirteen different authors, presenting special aspects of cytology from the standpoints of specialists in different fields. Their work is valuable, but, like many botanical textbooks, lacks unity, and does not give the comprehensive treatment of the subject which was so stimulating in the earlier editions of *The cell in development and inheritance*, and which has been so greatly extended and improved in the present edition.

A glance at the table of contents of the third edition shows numerous topics which were practically unknown in 1900. These newer subjects, especially the

[1] WILSON, E. B., The cell in development and inheritance. 3d ed. 8vo. pp. xxxvii+1232. *figs.* 529. New York: Macmillan. 1925.

chondriosomes of both plants and animals, are described in detail and thoroughly illustrated. The feature which most impresses the older investigator, however, is the changed point of view in treating subjects like the chromosome, centrosome, fertilization, and embryogeny, showing the great advance of knowledge in this direction during the last few decades.

Throughout the book the treatment shows the perspective and caution which comes from increasing knowledge of the facts, and this is nowhere more evident than in descriptions and discussions of chromosomes. Here much more attention is given to plants than in the previous editions. The presentation is eminently fair, although it is plain that the author does not agree with the theories of the Gregoire school in regard to the structure of the chromosome. They do not believe in the existence of the chromomere as a definite entity, but WILSON believes that "such skepticism cannot be maintained in view of the positive results of recent careful studies." Nevertheless, the reviewer must confess to an increasing skepticism in regard to the universal existence of chromomeres. If both theories are correct, so that in some forms the chromosome may consist of linin and chromomeres, while in other forms it consists of only homogeneous, vacuolated chromatin, theories of heredity will be more difficult to formulate.

An antithetic alternation of generations in animals, with the egg and three polar bodies (polocytes) as one generation and the soma as the other, is still denied; but the discussion, which is intended to prove that an antithetic alternation of generations, like that in plants, does not exist in animals, shows that the author has studied botanical literature and progressed far beyond his colleagues in familiarity with botanical problems. Another advance like this might give us an account of antithetic alternation of generations in animals, and give an interpretation of the polar bodies which would seem logical to a botanist. As the account stands, our viewpoint in regard to alternation of generations in plants is not yet fully appreciated, as is indicated in the statement that "in *Fucus vesiculosus* there is only one generation, the diploid, as in animals." We agree that in *Fucus*, and better still in *Plumbagella*, the situation is the same as in animals; but the origin and fundamental nature of alternation of generations in plants demands two generations at the extremes, like *Spirogyra*, *Fucus*, and *Plumbagella*, just as in easily recognized intermediates like *Cutleria*, mosses, and ferns. We agree, absolutely, that there is no genetic relation between alternation of generations in plants and animals, but we believe there is a case of parallel development such as is seen in the attainment of heterospory by unrelated phyla of plants.

The treatment of the reduction divisions in both animals and plants will be particularly helpful to botanists, because they are put into easy contact with the zoological situation by clear diagrams, in addition to a profusely illustrated text. In this connection, there is an extended presentation of differentiation among chromosomes, the relation of chromosomes to the determination of sex, and to various phases of heredity.

The comprehensive treatment of the embryology of animals should be sup-

gestive to botanists, for the embryology of plants has never been so thoroughly worked out, and what results have been obtained have not been so thoroughly organized.

The glossary will doubtless be welcome, even to zoologists, and for botanists it is indispensable. The double subject and author index is extensive, and has numerous catchwords which will save time in looking up references. A special bibliography is given at the end of each chapter, and in addition there is a general list of literature occupying 55 pages. The number of papers cited was not counted, but was roughly estimated to be about 2,000 citations.

On the whole, the book will stimulate and facilitate botanical as well as zoological research, and the reviewer predicts that for a long time it will hold its place as the standard work on cytology.—C. J. CHAMBERLAIN.

Colloid chemistry of protoplasm

A very readable discussion of the general and special colloidal constitution and behavior of protoplasm has been prepared by LEPESCHKIN,[2] whose contributions to protoplasmic behavior have won him recognition in this field. The volume is no. 7 of the series of *Monographien aus dem Gesamtgebiet der Physiologie der Pflanzen und der Thiere*. The introductory section deals with the general nature of hydrophobe and hydrophile colloidal state, and the problems of diffusion, osmosis, electrical charge, adsorption, viscosity, surface tension, and changes in the degree of dispersion. It also considers the general colloid chemistry of the proteins and lipoids.

The second section, part I, presents a picture of the general chemistry of protoplasm as a mixture of substances in colloidal state. Consideration of state of aggregation of protoplasm, nucleoplasm, plastids, and fibrillae leads to the conclusion that in the active state, protoplasm and the plastids form a colloidal solution, not a gel, except in the case of nerve and muscular fibrillae. These fibrillae are gel structures. The general colloidal structure of the living material, disperse phase and dispersion medium, receives detailed consideration, and some attention is given to the problems of viscosity, osmotic pressure, and electrical phenomena in the living protoplasm. Reversible and irreversible phenomena are described. With reference to the problem of osmotic pressure LEPESCHKIN finds the membrane idea of osmosis useless. In this connection it is obvious that LEPESCHKIN, like many others, has his attention centered too much upon the non-diffusible elements, which serve only to produce continuing unbalanced distribution of water, and not enough upon the diffusible water which is mainly responsible for the development of pressures, whether a membrane is present or not.

The final section, part II, presents the special features of protoplasmic colloidal behavior, those things in which protoplasm differs from other colloidal

[2] LEPESCHKIN, W., Kolloidchemie des Protoplasmas. 8vo. pp. xii+228. Berlin: Springer. 1924.

systems. He takes up first the chemical constitution of the dispersion medium (which is not water, but a substance in which water dissolves), and the disperse phases of cytoplasm, nucleus, and chromatophores. Then follows a discussion of the effects of high and low temperature changes, mechanical agencies, desiccation, light, and electric currents. The final pages consider the influence of electrolytes, including the neutral salts, salts of trivalent metals, heavy metals, acids and bases, and of non-electrolytes such as the indifferent narcotics, alcohol, acetone, chloroform, benzol, ether, etc., upon the colloidal state of these phases. The nature of narcosis receives very brief consideration.

Those who have kept up with the research will no doubt consider this book an elementary treatise. It is simply written, and for beginning students of protoplasmic behavior it presents a timely summary of the great advances which are being made in development of biological theories, and in the understanding of the conditions and processes of life, through the application of colloidal physics and chemistry to the investigation of the living substances of plants and animals. —C. A. Shull.

The American oaks

Trelease[3] has published a notable contribution to our knowledge of the taxonomy of oaks, the result of many years of investigation. His original plan was to study the species of tropical America, those of the United States having been sufficiently presented. Finally, however, he included all of the American oaks, those of the United States being treated in a more summary way than the others. Naturally the treatment of old segregates and the publication of new ones will attract the attention of taxonomists interested in a very puzzling genus. In consequence of the previous taxonomic tangles, the author has attempted to reorganize the material in a more satisfactory way.

The broad background of his conclusions was developed by a study of all of the types, which are reproduced in excellent photographic illustrations, by an investigation of the past history of oaks, and by a consideration of the development of the geographic areas involved. In this way he has been able to unify the nomenclature of fossil and living species, and to organize the species into natural groups, indicating in a chart the convergences and affinities among these groups.

There is a full description of the characters of the genus and their relative value in indicating relationships. Abnormalities are also included, as well as a list of American hybrids. A noteworthy item is the recognition of *Protobalanus*, as including "the intermediate oaks," and its probable ancestral relationship to the white and black oak groups. Some idea of the amount of material investigated may be obtained from the fact that 373 species are recognized, in addition to 32 varieties and 221 forms. For example, under *Q. stellata*, 14 forms and one

[3] Trelease, W., The American oaks. Mem. Nat. Acad. Sci. **20**:pp. 255. *pls.* 420. 1924.

variety are recognized. These numerous species, with their varieties and forms, are further organized into 131 natural groups.

This contribution will certainly be a standard reference in any study of the taxonomy of American oaks.—J. M. C.

The New England–Acadian shoreline

An attractive volume has recently appeared[4] that deals with the shores of the maritime provinces of Canada and those of New England. While the reviewer is not able to criticize the accuracy of the main physiographic portion of the book, he has found the material interesting, well organized, clearly presented, and well illustrated by means of photographs, maps, and diagrams. It is necessarily of decided interest to ecologists studying the shore vegetation, and more especially because, in addition to describing the physical substrata for shore plants, it presents a rather detailed study of salt marshes. The different theories accounting for the origins of such marshes are discussed, and the theory propounded by MUDGE is favored, that is, that the marsh is usually a record of a progressive subsidence of the land during the time of its formation. There seem to be three rather distinct types of such marshes, designated from the regions of their development: (1) New England type; (2) Fundy type; and (3) Coastal plain type. The first type usually exhibits three distinct, rather broad zones characterized by *Juncus Gerardi*, *Spartina patens*, and *S. glabra* respectively, and is developed upon a substratum consisting largely of salt marsh peat. The second type has much more silt in its substratum, and much narrower strips of vegetation. The third type is rather closely related to the second, but differs somewhat from it in the character of the silt and in the floristic composition of the vegetation. Variations from these types are discussed and many examples are cited. Extensive bibliographies are appended to each chapter.—GEO. D. FULLER.

MINOR NOTICES

Phytoplankton of Wisconsin.—The Wisconsin Geological and Natural History Survey has been conducting an important investigation of the phytoplankton of the inland lakes of Wisconsin. The first publication of the results appeared in 1920, including all plankton algae except desmids and diatoms. The second part has just appeared,[5] presenting the Desmidiaceae. The species are amply described and illustrated by 37 excellent plates, resulting in a notable contribution to our knowledge of this group. The volume presents 22 genera of Desmidiaceae, including 159 species. Much the largest genus is *Staurastrum*, with 59 species, 5 of which are described as new, the only new species in the volume, although there are numerous new varieties. The next largest genera are *Cosmarium* (15 species), *Micrasterias* (15 species), *Euastrum* (11 species),

[4] JOHNSON D., The New England–Acadian shoreline. 8vo. pp. xx+628. *figs.* 273. New York: John Wiley & Sons. 1925. $8.50.

[5] SMITH, G. M., Phytoplankton of the inland lakes of Wisconsin. Part II. Desmidiaceae. Wis. Geol. and Nat. Survey, Bull. 57. pp. 227. *pls.* 52–88. 1925.

Arthrodermus (11 species), and *Closterium* (10 species). The remaining 38 species are distributed among 16 genera. This contribution is also issued as a Bulletin of the University of Wisconsin.—J. M. C.

NOTES FOR STUDENTS

Taxonomic notes.—BERGER[6] has made a very critical taxonomic study of *Ribes* and *Grossularia*, including wild species and forms under cultivation, involving numerous hybrids. He recognizes 67 species of *Ribes*, 4 of which are new, and 52 species of *Grossularia*, 3 of which are new. In addition to the new species, there are many new combinations.

BISBY and BULLER[7] have published a list of the known fungi of Manitoba, which is announced as very incomplete. The list includes 574 species, distributed as follows: Myxomycetes 34, Bacteria 8, Phycomycetes 20, Ascomycetes 62, Fungi Imperfecti 38, Smuts 20, Rusts 80, Polypores 63, Agarics 108, Thelephoraceae 36, other Basidiomycetes 57, Lichens 48.

The third part of THAXTER'S monograph of Laboulbeniaceae has just appeared.[8] The two preceding parts have been reviewed in this journal.[9] Since the publication of the second part, the author has published nine preliminary communications appearing in the Proceedings of the American Academy, and including more than 400 species in 14 genera. The major part of the present contribution includes 4 genera of the Dimorphomyceteae; 77 species of *Dimeromyces* parasitic on Coleoptera, Diptera, Orthoptera, and Acarini; and the genus *Chitomyces*, in which 54 species are considered, 9 of which are described as new. This contribution, as the preceding ones, is a model of painstaking and exact work, and further emphasizes the wealth of material in this unique group.

WILDEMAN[10] has investigated the available African material of *Tephrosia*, recording 30 species, 9 of which are described as new.

The Laboratory of Agriculture of San Salvador has published a list of the plants of El Salvador (Central America), under the editorship of STANDLEY and CALDERÓN,[11] with the collaboration of 20 specialists in different groups, and under the auspices of the Smithsonian Institution. Although the list is made up chiefly of angiosperms, there are also a considerable number of fungi, lichens, liverworts, mosses, and ferns.—J. M. C.

[6] BERGER A., A taxonomic review of currants and gooseberries. N.Y. State Agric. Exp. Sta. Tech. Bull. 109. pp. 118. 1924.

[7] BISBY, G. R., and BULLER, A. H. R., Preliminary list of Manitoba fungi. Trans. Brit. Mycol. Soc. **8**:91–109. 1922.

[8] THAXTER R., Contribution toward a monograph of the Laboulbeniaceae. Part III. Mem. Amer. Acad. Sci. **14**:315–426. *pls. 12*. 1924.

[9] BOT. GAZ. **23**:216. 1897; **47**:156. 1909.

[10] WILDEMAN É. DE, Observations sur des espèces Africaines du genre *Tephrosia*. Pers. Bull. Soc. Roy. Bot. Belgique **57**: 114–129. 1925.

[11] STANDLEY, P. C., and CALDERÓN S., Lista preliminar de las plantas de El Salvador. pp. 274. 1925.

Carbon dioxide from roots.—From a series of experiments on buckwheat, sorghum, soy beans, cow peas, cotton, and velvet beans grown in soil and sand cultures, PARKER[12] presents evidence to show that CO_2 production is not related to the feeding power of these plants for calcium, magnesium, phosphorus, or potassium. The measure of feeding power for a given element is taken as the relative abundance or scarcity of the element in the composition of the plant. The studies included: (1) the influence of the crop on the CO_2 content of the soil air; (2) the effect of continuous aspiration of the cultures on the feeding power of the plants; (3) the total quantity of CO_2 excreted by the roots; (4) the amounts of the different elements absorbed per gram of CO_2 given off. Cow peas excreted more CO_2 than any of the other plants used, and buckwheat gave off very little CO_2, although it had the greatest feeding power. Cotton ranked second in feeding power and sorghum last. Sorghum, soy beans, and cotton were very similar in CO_2 production. The removal of CO_2 by continuous, rapid aspiration did not influence the composition of the plants. For each gram of CO_2 excreted, buckwheat absorbed 41.5 mg. of calcium, soy beans 21.2 mg., cow peas 12.7 mg., and sorghum 5.0 mg.—R. B. DUSTMAN.

Tertiary flora.—KUBART[13] has published a contribution to our knowledge of the Tertiary flora. The University of Graz has had a succession of notable paleobotanists, beginning with UNGER, who was followed by ETTINGSHAUSEN, whose laboratory and collections are now in charge of KUBART. The proximity of Tertiary plant deposits has influenced paleobotanical studies at Graz, and a practical side has been given the development of the subject by the present need of more information concerning the lignite beds in Austria.

In the present contribution a careful comparison is made between Tertiary woods and such living woods as can be traced to the Tertiary, as *Sequoia*, *Taxodium*, and *Pseudotsuga*. Not only is the morphological side of the study carried out in detail, but also, by making comparisons with recent swamps in the southern part of the United States and in Mexico, the geological aspect of the origin of Austrian lignite deposits is investigated. Here the author comes to the conclusion that there is no necessity to accept the theory that the Austrian lignite deposits originated only in swamp lands. This may have been the case in many instances, especially at the beginning of the filling out of a land depression, but KUBART believes that the climatic factors of that time favored the preservation of plant material without the formation of extensive swamps in the large *Sequoia* forests of the Tertiary.—A. C. NOÉ.

[12] PARKER, F. W., Carbon dioxide production of plant roots as a factor in the feeding power of plants. Soil Science **17**:229–247. 1924.

[13] KUBART, B., Beiträge zur Tertiarflora der Steiermark nebst Bemerkungen über die Entstehung der Braunkohle. Arbeiten Phytopal. Lab. Univ. Graz I. pp. 62. *pls.* 2. 1924.